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Biotin requirement of the guinea pig

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BIOTIN REQUIREMENT OF THE GUINEA PIG

by

Gertrude LeAnn Borchers

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
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DOCTOR OF PHILOSOPHY

Major Subject: Nutrition

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INTRODUCTION

The guinea pig has been used as a laboratory animal since the early part of the 20th Century, but without an adequate knowledge of its nutritional requirements. This inadequacy has in turn limited the value of the guinea pig, so in spite of its introduction by Holst and Fröhlich in 1907 for their studies of ship beri-beri and scurvy, the rat, whose qualitative and quantitative nutritional requirements have been more satisfactorily determined, has become more popular as a laboratory animal. During the years since 1907, relatively few attempts were made until recently to determine the nutritional requirements of the guinea pig. Hence limited information has been available about the necessity of many vitamins, including biotin, for growth and survival of the guinea pig.

In his review of the nutritional requirements of the guinea pig Mannering (1949) listed four qualities in the guinea pig which have made it less desirable than the rat as a laboratory animal. First, the guinea pig is more specialized in its dietary requirements than the rat, hence the development of a purified diet for the guinea pig has been more difficult than for the rat. Purified diets, however, are a prerequisite for many nutritional studies. Second, guinea pigs are extremely susceptible to infections. Infections cause weight loss, poor food consumption, and death, all of which may be entirely unrelated to experimental diets.

Third, the guinea pig is a notorious coprophagist. This behavior is most disturbing when attempts are made to assess its qualitative and quantitative needs of the B vitamins since feces contain variable amounts of the B vitamins, but generally are a very rich source. Fourth, the guinea pig lacks genetic uniformity with regard to both birth weight and growth rate. These four qualities lead to difficulties in interpretation of results and also may account for differences in results between laboratories.

In his review Mannering (1949) also stated that the literature revealed only meager and often inconclusive evidence for the production of deficiencies of various members of the vitamin B complex in guinea pigs. While production of deficiencies of most vitamins of the B complex had to await the development of a purified diet, efforts to obtain a biotin deficiency occurred much earlier. Lease et al. (1937) attempted to produce a condition similar to the egg white injury of rats in several other species. Only inconclusive results were observed in the guinea pig. Changes included rapid loss of weight, unkempt appearance, and loss of hair, but such changes might well have been due to toxicity of egg white since an extract of liver which cured the symptoms in other species failed to do so in the guinea pig.

Reid (1953, 1954) in a more recent study omitted biotin from a purified diet and found no differences in growth and

survival between groups receiving biotin and those which did not. She therefore concluded that a dietary source of biotin was not necessary for the guinea pig.

Reid (1953, 1954), however, was concerned only with whether or not a dietary source of biotin was needed under normal conditions of growth when all other known requirements were included in the diet and no attempt was made either to suppress the synthesis of biotin by microorganisms in the gastrointestinal tract or to prevent its absorption. Biotin produced by microorganisms in the gastrointestinal tract and available for absorption by the guinea pig may have masked the need of the guinea pigs for a preformed source of biotin.

Biotin is so widespread in nature that the development of a deficiency using a diet composed of natural food stuffs is most unlikely. As late as 1949 a satisfactory synthetic diet was not available, but recent workers (Reid and Briggs, 1953) have developed a reasonably satisfactory synthetic diet which allowed an average daily weight gain of 7.1 grams. Cannon et al. (1946) suggested that the normal weight gain rate was 7-8 grams per day when the guinea pig was between the ages of 2 and 8 weeks and when the diet consisted of natural food stuffs. The diet developed by Reid and Briggs (1953) contained an excess of each of the known B vitamins and, in their hands, was capable of maintaining the guinea pigs in good health for 150 days. It allowed reproduction to

occur, but was not as satisfactory for reproduction as it was for growth.

The use of a satisfactory synthetic diet for guinea pigs may lead to the finding of unidentified factors, to the clarification of the status of known factors, and to studies of vitamin interrelationships of interest to the whole field of nutrition. The fact that the guinea pig is more specialized in its dietary requirements is, therefore, an asset rather than a detriment. With the advent of a synthetic diet, identification of the anti-stiffness factor (Van Wagtendonk and Wulzen, 1950) and guinea pig factor three (Woolley and Sprince, 1945) may be simplified. Also the need of the guinea pig for vitamin P, flavone glycoside, (Rusznayak and Szent-Györgyi, 1936) and strepogenin (Woolley, 1941) can be evaluated. Since the guinea pig is the only small laboratory animal requiring ascorbic acid, studies of the biochemical role of ascorbic acid and its relationships with other nutrients may be aided by an adequate knowledge of the guinea pig's nutritional requirements.

The present study had two objectives. The first was production of biotin deficiency in the guinea pig, the second, the detection of metabolic changes occurring as a result of the deficiency. The most common method for developing biotin deficiency in rats has been based on powdered egg albumin in the diet. However, Lease et al. (1937) has suggested that egg

white is possibly toxic to the guinea pig. When fed powdered egg albumin as 66 per cent of the diet (the same as that used for rats in her study) guinea pigs declined steadily in weight after the second day and died in 5 to 10 days. The response was not quite as drastic if egg white was gradually increased to 50 per cent of the diet. As this thesis was being prepared Coots et al. (1959) reported success in developing biotin deficiency in adult guinea pigs on a diet containing 30 per cent dried egg white. However, weanling animals failed to adjust to the diet and died within 10 days. Other possible methods than the use of egg white for producing the deficiency include (1) the use of a bacteriostatic agent such as aureomycin or a sulfonamide to prevent the synthesis of biotin by microorganisms in the gastrointestinal tract, (2) the use of an analog to act as a competitive inhibitor to biotin, and (3) the use of isolated avidin to prevent absorption of biotin.

Several metabolic processes requiring biotin have been identified in microorganisms, but studies of the role of biotin in higher animals have been concerned mostly with the description of gross changes which occurred as a result of biotin deficiency. In microorganisms biotin has been associated with carbon dioxide fixation, decarboxylation, deamination of aspartic acid, threonine, and serine, synthesis of aspartic acid, synthesis of oleic acid, and carbohydrate

oxidation.

This work was done then to determine the need for biotin in the nutrition of the guinea pig with the hope that the findings would contribute to making the guinea pig a more satisfactory laboratory animal for the investigation of nutritional problems and that the observations on metabolic changes occurring during the deficiency would contribute to an understanding of the role of biotin in higher animals.

REVIEW OF LITERATURE

Biotin and Egg White Injury

"Egg white injury syndrome" in rats was known before isolation and identification of the vitamin, biotin. The syndrome was observed by Boas (1924a) during development of a purified diet for calcium studies. Prior to this time Batemann (1916) had reported the undesirability of egg white as a source of protein for several species including rats and dogs. He had attributed the undesirability to the presence of an antitryptic factor.

Boas was interested in determining the nature of "egg white injury" and succeeded in finding certain foods such as liver and kidney which protected the animal from the symptoms. She called the substance found in these foods protective factor X (Boas, 1927).

A second group of workers (Lease and Parsons, 1934), interested in the nature of "egg white injury", called a water soluble factor which they obtained by extraction of a commercial liver preparation "the factor protective against egg-white injury".

György and coworkers (1940) who were also working on the nature of "egg white injury" named a protective substance vitamin H because of the protection it gave from skin (Haut) symptoms.

During this time a growth factor for microorganisms was isolated from egg yolk in the form of a crystalline methyl ester by K^ogl and T^onnis (1936). They called the growth factor biotin. Biotin was later shown to be identical with Coenzyme R (Allison et al., 1933) by West and Wilson (1939) and with vitamin H by duVigneaud et al. (1940) and Gy^orgy et al. (1940).

Biotin Deficiency in Different Species

Boas (1927) was one of the first to describe "egg white injury" syndrome in rats. Her classical description follows:

Rats grow well and are usually in good health for from 2-3 weeks. Then red scaly patches appear at the corners of the mouth, the coat becomes rough and sticky and the long hairs fall out. The fur on the abdomen shows at first a characteristic rubbed appearance followed by development of bald areas. Meanwhile the red patches spread to other parts of the body and the picture is one of an eczematous dermatitis. There are even skin haemorrhages in severe cases. The region round the mouth is always the most seriously affected though there is often such marked blepharitis that the eyes are closed. The loss of hair is often extensive. In a few cases redness of the feet has been seen but this does not usually occur. The rats always have a distinctive somewhat musty smell probably due to some constituent of the urine. The body weight remains stationary for a week or two, but falls slowly during the second stage of the disease. This is reached about 2-3 weeks after the first signs of deficiency. To the dermatitis, symptoms of nervous upset are now added. There is a pronounced spasticity of the limbs, particularly of the hind legs, and the back is arched. The rat assumed in many cases a kangaroo like posture. (Boas, 1927, pp. 712-713)

The animals eventually died, death being preceded by a rapid weight loss and cyanosis. Upon autopsy Boas (1927) noted an absence of fat and that the skin was infiltrated and vascularized. The other organs were seemingly normal.

In addition to the deficiency in rats, biotin deficiency has been produced in a number of other species. Wilson et al. (1949) produced biotin deficiency in mice with diets containing 20 per cent dried egg albumin, both with and without sulfaguanidine included as 0.5 per cent of the diet. They observed most of the symptoms described in the rat such as cessation of growth and loss of hair, but did not obtain the accumulation of the brownish seborrhal material on the skin. When black mice of the C₅₇ strain were deficient, their fur became rusty or gray and areas of the skin became denuded. After intramuscular injection of 2 mcg. of biotin, while animals were still on the deficiency-producing diet, new hair growth appeared on denuded spots. The hair in some cases was gray at first, but it eventually assumed normal color. Nielsen and Black (1944) produced biotin deficiency in mice using a synthetic diet containing no egg white but containing 0.5 per cent sulfasuxidine. The deficiency was characterized by severe alopecia.

Emerson and Keresztesy (1942) noted depigmentation of the fur of black rats fed a deficiency-producing diet. Also present was exfoliative dermatitis of the abdomen which

developed into a general alopecia, "spectacle eye", and abnormal gait. When biotin was given, complete recovery from all symptoms except "spectacle eye" and graying was accomplished.

Biotin deficiency was produced in pigs by feeding a purified diet containing 30 per cent desiccated egg white (Cunha et al., 1946). After receiving the diet for 2 1/2 weeks the pigs lost hair from their hind quarters and eventually from the back and sides of their bodies. Dermatitis developed over the entire body at the same time as hair loss. During the fifth week cracks in the skin on the feet and spasticity of the hind legs appeared. None of these symptoms appeared in control animals receiving the same diet but given 100 micrograms of crystalline biotin by intramuscular injection. The biotin-deficient pigs also grew more slowly than their controls.

Lehrer et al. (1952) obtained a somewhat different syndrome with 48 hours-old suckling pigs fed a biotin-deficient, purified diet which did not contain egg white. A dermatitis which first appeared on their ears, neck, shoulders, and tail eventually covered the entire body. General alopecia developed and eventually ulcerated areas appeared on their hind quarters and belly. Severe cracks which developed in the skin on their feet made walking difficult. An inflammation

of the mucous membranes of their mouths was part of the syndrome, but the animals continued to eat normally and to weigh approximately the same as controls. Post mortem studies revealed no macroscopic abnormalities other than the skin changes.

Ansbacher and Landy (1941) were successful in producing a biotin deficiency in another species, the chicken, with the use of only a low biotin diet (0.1 mcg. biotin per gram of diet). Chicks developed dermatitis on their toes and feet, at the corners of their beaks, on their combs, and occasionally on their eyelids. When 1.25 mcg. of either biotin or biotin methyl ester were fed each day, mild skin symptoms present in some chicks were cured, but larger amounts were needed to effect cures of severe skin symptoms present in other chicks.

Other workers (McElroy and Jukes, 1940) feeding egg white to chickens obtained more acute symptoms than Ansbacher and Landy (1941). McElroy and Jukes noted that the most outstanding symptoms of the deficiency produced with dried, uncooked egg white were keratinization and fissuring of the feet. In addition to dermatitis, Jukes and Bird (1942) reported that symptoms of perosis developed in 50 to 70 per cent of the chicks. Perosis could be prevented by omitting egg white, by substituting cooked egg white, or by intramuscular injection of 0.54 mcg. biotin per bird per day.

Birds receiving biotin also had superior feathering to those not receiving biotin.

Waisman et al. (1945) reported a chronic biotin deficiency in monkeys after they received 1.3 to 3.4 mcg. biotin from dietary sources daily for 15 to 24 months. The deficiency was characterized by loss of hair and loss of hair color, both of which could be restored by supplementing the diet daily with 20 mcg. of crystalline biotin methyl ester. These workers also produced a chronic biotin deficiency in monkeys by incorporating 10 per cent dried egg albumin into their diet. The deficiency produced in monkeys by this diet resulted in severe dermatitis around their face, hands, and feet. Their eyes watered, became red, and their eyelids became encrusted. Some loss of hair color was noticed in monkeys maintained on the egg white diet for 4 months. The biotin-deficient monkeys also seemed more susceptible to infections than their controls.

Lease et al. (1937) reported biotin deficiency in monkeys fed 20 per cent dried egg white in their diet for 2 months followed by a diet containing 40 per cent dried egg white. No changes were noted for 3 weeks after raising the egg white to 40 per cent of the diet but thereafter a progressive roughening and thinning of the hair occurred. Within 2 months the monkeys had bald areas on their tails and heads, had de-

creased their activity and sat hunched in their cages with their faces hidden. Their eyelids became red.

One attempt (Sydenstricker et al., 1942a) has been made to produce biotin deficiency in human subjects. Two men and two women were fed a diet containing a minimal amount of biotin and egg white equal to 30 per cent of their calorie intake. A fine scaly condition of the skin developed within 4 weeks, but did not persist. During the seventh week the subjects began to show a grayish pallor of their skin and mucous membranes. Dryness and scaliness of their skin reappeared at this time and the subjects developed muscle pains, hyperaesthesia, lassitude, and mental depression. These symptoms disappeared when daily doses of 150 to 300 mcg. of biotin were given by injection. A continuation of the report of this experiment appeared later the same year (Sydenstricker et al., 1942b). In addition to the above symptoms, a definite decrease in hemoglobin concentration, volume of packed red cells, and urinary biotin occurred. The urinary biotin excreted in 24 hours decreased from 29 to 62 micrograms during the control period to 3.5 to 7.3 micrograms daily.

Whereas Woolley and Sprince (1945) concluded that the addition of biotin and para-aminobenzoic acid to a semi-synthetic diet for guinea pigs resulted in better survival,

there have been no reports of a biotin requirement by this species until Coots et al. (1959) published results of experiments establishing the need for biotin by the guinea pig. Earlier, Lease et al. (1937) attempted to produce a deficiency using egg white. With 66 per cent egg white in the diet, guinea pigs declined rapidly in weight, became prostrate, had convulsions, and died in 5 to 10 days. If egg white was introduced stepwise to 50 per cent, the guinea pig appeared to tolerate the egg white, but became inactive and unkempt in appearance. The latter was partly due to egg white sticking to their fur. No baldness or sore areas were observed except for transient loss of hair and slight increase in redness of their lower lip and toes. The authors were uncertain whether the transient loss of hair might not have been due to the embedding of diet in the hair. Growth was slow, but otherwise manifestations were vague. Since a potent extract prepared from a commercial liver powder cured the egg white injury symptoms in other species but did not improve growth rate or the unkempt appearance of the guinea pigs, the authors felt there was some doubt whether the symptoms were due to biotin deficiency or to toxicity of egg white. Despite the uncertainty, the experiment has often been cited as indicating a need for biotin by the guinea pig.

Reid (1954) found no difference in growth and survival

when she compared guinea pigs on a synthetic diet, some with and some without a dietary supply of biotin. In her monograph, Reid (1958) reported that her findings did not exclude a biotin requirement for the guinea pig, since the need, if any, was probably satisfied by intestinal synthesis.

Methods of Producing a Biotin Deficiency

The use of synthetic diets

While it has been possible in the case of the chicken (Ansbacher and Landy, 1941), pig (Lehrer *et al.*, 1952), and monkey (Waisman *et al.*, 1945) to produce a biotin deficiency using a purified diet, little success has been obtained with other species unless diets contained uncooked egg white as a source of protein. Wright and Welch (1944) were able to depress the amount of biotin in the liver, but found no other evidence of a deficiency in rats after several months of feeding a synthetic diet low in biotin. Casein was the source of protein. Skegg and Wright (1946) confirmed these findings and indicated that rats fed a purified diet containing no biotin grew normally. Reid (1953, 1954) using a purified diet found no significant difference between weights of guinea pigs lacking a dietary supply of biotin and those receiving biotin.

Cannon and Emerson (1939) reported one of the first

attempts to develop a purified diet for guinea pigs. They took into consideration the differences of habit and anatomy which makes the form and consistency of the diet more important to guinea pigs than rats. In their diet yeast was the source of B vitamins. Two of five guinea pigs receiving the diet died after a month and the remaining three were weak and had poor muscle tone. Addition of 30 grams of fresh lettuce daily to the diets of these three guinea pigs allowed them to continue on experiment for 145 days, during which time they gained an average of 344 grams. The first use of crystalline B vitamins in a purified diet resulted in poor growth and survival (Woolley, 1942; Woolley and Sprince, 1945; and Cannon *et al.*, 1946). Woolley (1942) postulated that the guinea pig needed three unknown factors. Two of these have been identified subsequently by Woolley and Sprince (1945). Guinea pig factor 1 was identified as folic acid and the requirement for guinea pig factor 2 was satisfied by raising dietary casein from 18 to 30 per cent (or adding arginine, cystine, and glycine to 18 per cent casein) plus cellulose. Guinea pig factor 3 has not been identified. Although biotin and para-aminobenzoic acid increased survival time, neither were identical with guinea pig factor 3. On the other hand, Woolley (1941) believed that guinea pig factor 3 resembled strepogenin.

Booth et al. (1949) made two contributions by recognizing the importance of bulk in synthetic diets and by improving their mineral content with the addition of ash from alfalfa. When Roine et al. (1949) replaced alfalfa ash with 0.5 per cent magnesium oxide and 2.5 per cent potassium acetate, successful growth, 7.2 grams daily, was observed for five guinea pigs. Both groups of workers included biotin in their diets.

The most recent synthetic diet, published in 1953 by Reid and Briggs, supported weight gains of 7.1 grams per day. It contained large amounts of the known B vitamins including biotin. Omission of a single vitamin from this diet resulted in deficiencies of thiamine, riboflavin, folic acid, choline, pantothenic acid, pyridoxine, and niacin (Reid, 1953, 1954; Reid and Briggs, 1954). Vitamin B₁₂, inositol, para-aminobenzoic acid, and biotin were not essential for satisfactory growth.

The use of bacteriostatic agents

Although feeding a purified diet low in biotin does not result in a biotin deficiency in some species, a preformed source of the vitamin may still be necessary. Preformed sources of biotin may be available as a result of synthesis of biotin by microflora in the gut, the vitamin being either

absorbed directly or obtained through ingestion of feces.

Several groups (Nielson et al., 1942; Skegg and Wright, 1946; and Ham and Scott, 1953) have reported that the amount of biotin in feces was considerably higher than that in the diet and the total amount of biotin excreted in feces and urine was higher than the biotin consumed in the diet. For instance, Nielson et al. (1942) reported that rats fed a purified diet excreted from six to 12 times as much biotin as they obtained in their food. Steenbock et al. (1923) noted poor growth in rats kept in tubular cages to prevent coprophagy, and found that they needed much higher amounts of B vitamins in their diet. Barki et al. (1949) produced a growth response in rats kept in tubular cages by feeding them additional biotin. On a purified diet containing only small quantities of biotin, no symptoms of biotin deficiency other than restricted growth were observed although amounts of biotin in the liver were reduced.

When a synthetic diet containing no biotin was fed to germ-free rats (Luckey et al., 1955), they gained weight for 4 months but during the fifth month began to lose weight and developed alopecia, spectacle eyes, and open ulcers on their shoulders, head, and ears. Biotin supplements were effective in prevention of all of these symptoms.

Bacteriostatic agents have been used to suppress the

growth of microflora in the gut. Mickelsen (1956) has suggested that whether an antibiotic or some other antimicrobial agent in the diet stimulates, depresses, or has no effect on the growth of an animal depends to a large extent upon the kinds of microorganisms present in the intestinal tract.

Effects reported when aureomycin has been added to the diet fed to guinea pigs have been variable. Roine and Ettala (1952) and Roine et al. (1955) reported rapid loss of weight followed by death of guinea pigs receiving aureomycin. Jukes (1955) in reviewing the role of antibiotics in nutrition acknowledged Roine's findings, but also referred to a private communication which concluded that the inclusion of aureomycin in diets controlled infections ordinarily prevalent in colonies and allowed good results as far as growth and survival were concerned.

Limited information is available in the literature as to whether inhibition of microorganisms by aureomycin affects synthesis of biotin in the gastrointestinal tract or not. When Halevy et al. (1955) included aureomycin in the diet, the amount of biotin in urine, caecum contents, and liver was not affected.

Workers have reported less variable findings with sulfasuxidine in the diet than with aureomycin. According to Mickelsen (1956) contradictory results obtained with anti-

biotics were due to the wide spectrum of bacteria inhibited by the antibiotics. Sulfonamides inhibit a more narrow spectrum of bacteria than antibiotics.

The use of sulfasuxidine, since it is so poorly absorbed from the intestinal tract, has been an extremely popular method for the production of biotin deficiency in rats. Only a limited number of the studies, representing experiments using different amounts of sulfasuxidine in the diets fed to rats, will be mentioned here. Barki et al. (1949) reported that the concentration of biotin was 0.82 mcg. per gram of fresh liver in a control group compared with 0.25 mcg. in a group receiving 0.5 per cent sulfasuxidine. They did not report whether any deficiency symptoms were present. Daft et al. (1942) used 1 per cent sulfasuxidine in the diet and obtained a dermatitis which was very similar in appearance to that produced by egg white and which could be prevented or successfully treated with crystalline biotin. Other changes not previously reported as characteristic of a biotin deficiency also occurred, but no explanation for their occurrence was given. By using 2 per cent sulfasuxidine Halevy et al. (1955) were able to depress growth and the amount of biotin in the urine, caecum contents, and liver. Skegg and Wright (1946) and Wright and Welch (1944) also observed smaller amounts of biotin in the livers of rats receiving sulfasuxi-

dine than those which did not receive sulfasuxidine. Total excretion of biotin in urine and feces was reduced 50 per cent when rats were fed 2 per cent sulfasuxidine (Ham and Scott, 1953). External symptoms characteristic of a biotin deficiency were reported for each study in which 2 per cent sulfasuxidine was added.

The use of analogs

Several analogs of biotin are known which inhibit growth of certain microorganisms. The effect is usually competitive and analog activity is measured in terms of its molar inhibition ratio. The molar inhibition ratio as defined by Dittmer and duVigneaud (1944) is the number of molecules of the inhibitor necessary to prevent the biological activity of one molecule of biotin.

An analog may be active for one organism but not for another and this is true for desthiobiotin, the biotin analog in which the sulfur atom is replaced by two hydrogens. Melville et al. (1943) found that desthiobiotin acted as a growth stimulant for Saccharomyces cerevisiae. This was confirmed by Dittmer et al. (1944), who also found that desthiobiotin acted as an anti-biotin for Lactobacillus casei. Rubin et al. (1945) established that its molar inhibition ratio was 17,000 for L. casei. A summary of the effects of desthiobiotin on various strains of microorganisms was compiled by

Lilly and Leonian (1944), but molar inhibition ratios were not presented. Another interesting finding was the fact that S. cerevisiae converted desthiobiotin to biotin (Dittmer et al., 1944).

Reports of the activity of desthiobiotin in higher animals are limited in number. Emerson in 1945 reported that biotin-depleted rats receiving desthiobiotin showed no improvement, and Rubin et al. (1945) found under similar conditions that desthiobiotin had an activity equivalent to about 0.1 to 0.01 per cent that of biotin.

The use of uncooked egg white

The most widely used method of producing a biotin deficiency has been the feeding of uncooked egg white in the diet. Hertz (1946) has written an excellent review of many early studies.

The nature of "egg white injury" was resolved by two different approaches. The first was the discovery of the protective factor biotin and the second was the identification of the specific substance in egg white capable of producing the syndrome. Eakin et al. (1940a) first suggested that the injury was not due to a direct toxin, but due indirectly to the action of the egg white in making biotin unavailable. Their suggestion was based on their findings of a low biotin content of tissues of chicks with "egg white

"injury" despite large amounts of biotin in the diet and in the feces. Further proof for this theory was provided by Eakin et al. (1940b) who demonstrated that in vitro addition of egg white to a medium containing biotin made biotin unavailable to yeast. One gram of egg white inactivated 2.2 mcg. of pure biotin. Since egg white inactivated biotin and since biotin concentration was low in the tissues of animals suffering from "egg white injury" they concluded that the constituent which rendered biotin unavailable was responsible for the "egg white injury". Eakin et al. (1940b, 1941) separated protein fractions of egg white and followed the concentration of the factor by yeast assay. The concentration involved acetone precipitation of egg white, followed by extraction of the curds with a solution of sodium chloride. When the extract was half-saturated with ammonium sulfate and acidified with acetic acid most proteins precipitated but the active material remained in solution. Further addition of ammonium sulfate precipitated the active material which was then suspended in water and dialyzed against water. By this procedure a concentration of the active compound 600 fold that found in dried egg white was obtained by Eakin and coworkers for which they proposed the name avidin. Woolley and Longworth (1942), using a similar method, succeeded in preparing a concentrate 15,000 times more active than egg white. Other procedures for the isolation of avidin have been

developed by Pennington et al. (1942) and by Dhyse (1954).

While there are many studies in which avidin was fed, in only one study was avidin isolated from egg white prior to feeding. György et al. (1941) isolated 231 mg. of avidalbumin concentrate from 100 grams of egg white and added the avidalbumin concentrate to 100 grams of dried cooked (inactivated) egg white. This dried cooked egg white and avidalbumin concentrate was fed as 30 per cent of the diet for a period of 2 weeks to rats suffering from egg white injury, during which time the condition of the rats continued to deteriorate. Rats suffering from egg white injury and receiving 30 per cent dried cooked egg white in their diet improved.

Morphological and Biochemical Changes

Follis, in his book The Pathology of Nutritional Disease (1948), presented an excellent introduction to the study of the morphological changes which have been observed during deficiencies of essential nutrients. He pointed out that virtually any dietary restriction led to some change in one or more tissues, but the problem which arose was whether the changes were due to specific restrictions or to complicating factors such as inanition. In simple caloric restriction, for instance, fatty tissue decreased markedly, atrophy of

lymphoid tissue reduced the size of lymph nodes and spleen, alopecia occurred and genital organs atrophied and were no longer capable of spermatogenesis and ovogenesis. Thus failure of the animal to eat or utilize its food efficiently may lead to many changes. Further, morphological changes in any acute nutritional deficiency were slight or absent in comparison with changes which occurred when the deficiency was chronic.

The manner in which individual organs were affected by reduced food intake was discussed by Jackson in his book Inanition and Malnutrition (1925) and by Keys et al., in their book The Biology of Human Starvation (1950). The pattern of weight loss of an organ was not necessarily the same as that of the body as a whole, but several generalizations were possible. The brain and spinal cord lost very little weight, but other soft tissues, particularly the liver and intestines, showed large losses. Loss of weight by the heart and kidneys was less proportionally than body weight loss. No generalization was possible for endocrine glands since their weight losses varied widely.

Dann and Darby (1945) suggested the following stages in the development of a deficiency disease. Decreased concentration of the vitamin in the blood is followed by diminished body stores and excretion, functional impairment, microscopic changes and finally gross anatomical changes.

The morphological changes which have occurred in biotin deficiency can be summarized as follows: symptoms usually present include exfoliative dermatitis, cessation of growth, alopecia, spectacle eye, and kangaroo posture. In view of the sequence of events listed by Dann and Darby, metabolic changes would have preceded development of these gross anatomical changes.

Although the role of biotin in the mammalian organism has not yet been elucidated, biotin has been found to be involved either directly or indirectly in several different areas of microbial metabolism. One of the first areas to be studied was the role of biotin in aspartic acid synthesis. Burk et al. (1941) and Koser et al. (1942) noted the sparing effect of aspartic acid on biotin requirement of yeast and promotion of yeast growth by aspartic acid when it replaced part of the biotin in the medium. Stokes et al. (1947) showed that certain lactic acid bacteria also appeared to require aspartic acid if the biotin supply was limited, but aspartic acid was not required with increased amounts of biotin.

Lichstein and Umbreit (1947b) observed a decrease in the deaminase activity for aspartic acid, serine, and threonine in biotin-deficient microorganisms. The addition of certain B vitamins (nicotinic acid, para-aminobenzoic acid, riboflavin, pantothenic acid, thiamine, folic acid, and

pyridoxal) other than biotin during aging (incubation at 37° C. in a vitamin-free medium) did not restore the activity. Biotin alone was nearly as effective as the B vitamins plus biotin in restoring deaminase activity.

The sparing action of aspartic acid caused workers to look for steps in the synthesis of aspartic acid for which biotin might be necessary. One such possibility was carbon dioxide fixation.

Lardy et al. (1947) in their study of the mechanisms by which biotin affected the synthesis of aspartate found that bicarbonate gave no growth response in yeast in a low biotin, aspartic acid-free medium, but did stimulate growth in the presence of a biotin-containing, aspartic acid-free medium. The inability of biotin-deficient microorganisms to synthesize aspartate, therefore, might be due in part to their inability to condense pyruvate and carbon dioxide producing oxalacetate which would then be converted to aspartate.

Lichstein and Umbreit (1947) found that biotin-deficient E. coli showed a reduced ability to produce carbon dioxide from aspartic, malic, or oxalacetic acid. The rate of decarboxylation of these acids was restored by the addition of biotin to the medium. Further, deficient E. coli, which ordinarily were stimulated by biotin, exhibited no such response when malate was oxidized if cyanide was present to

bind the oxalacetate formed. These observations would seem to locate the site of action of biotin at decarboxylation of oxalacetate.

Shive and Rogers (1947) found that α -ketoglutaric acid exerted a sparing effect on microbial requirement for biotin and concluded that biotin-deficient microorganisms could not form α -ketoglutaric acid. They postulated that oxalsuccinic acid was converted to α -ketoglutaric acid by reversal of the kind of carboxylation reaction whereby pyruvic acid is converted to oxalacetic acid.

Another study of the role of biotin in carbon dioxide fixation has recently been reported by Woessner *et al.* (1958). The action of biotin appeared to be in carboxyl transfer rather than in carbon dioxide fixation. The workers studied two enzymes which were required for the carboxylation of β -hydroxyisovaleryl coenzyme A (formed during the degradation of leucine). The steps in the process were suggested as follows:

1. $\text{CO}_2 + \text{ATP} \rightleftharpoons \text{adenyl carbonate} + \text{pyrophosphate}$
2. $\text{Adenyl carbonate} + \beta\text{-hydroxyisovaleryl coenzyme A} \rightleftharpoons \beta\text{-hydroxy-}\beta\text{-methylglutaryl coenzyme A} + \text{adenylic acid}$
3. $\beta\text{-hydroxy-}\beta\text{-methylglutaryl coenzyme A} \rightleftharpoons \text{acetoacetate} + \text{acetyl coenzyme A}$

In the liver extracts of a biotin-deficient rat the carbon dioxide-activating enzyme needed for the first reaction was

present in the same concentration as in liver extracts of normal rats, but -hydroxyisovaleryl coenzyme A carboxylase needed to complete step 2 was completely lacking in biotin-deficient rats.

Another area of biotin activity is in synthesis of oleic acid although the mechanism is unknown. Williams and Fieger (1946) reported that Lactobacillus casei was maintained successfully for over 4 months on an essentially biotin-free medium containing oleic acid. No synthesis of biotin could be demonstrated. No stimulation of growth with oleic acid was obtained when riboflavin-free, pantothenic acid-free, or nicotinic acid-free medium were used. Williams et al. (1947) confirmed the need for oleic acid by certain lactobacilli in a biotin-free medium. Broquist and Snell (1951) found L. arabinosus, L. casei, and S. faecalis required both aspartic acid and an unsaturated fatty acid (oleic acid) to permit growth in the absence of biotin while L. fermenti and C. butyricum required only the unsaturated fatty acid to permit growth. Ravel and Shive (1955) found biotin sulfone inhibited competitively biosynthesis of aspartic acid and oleic acid in L. arabinosus.

More recently Squires and Stumpf (1959) and Wakil and Ganguly (1949) have presented data indicating that biotin was present in isolated enzyme systems which converted ace-

tate into palmitic and oleic acids in the presence of HCO_3^- , ATP, TPNH, Mn^{++} , and CoA. Squires and Stumpf found avidin inhibited fatty acid synthesis, but inhibition could be prevented by preincubation of avidin with stoichiometric amounts of biotin in the enzyme system. Wakil and Ganguly suggested that the biotin-containing enzyme catalyzed the reaction of bicarbonate and acetate to form malonyl coenzyme A, an intermediate in fatty acid synthesis. The bicarbonate was subsequently removed, however, since radioactive bicarbonate was not incorporated into the fatty acids formed.

Biotin also has a role in carbohydrate metabolism of yeast. Moat and Lichstein (1954) reported that when biotin was added to a washed cell suspension of biotin-deficient yeast the rate of fermentation and oxidation of glucose by the cells was stimulated. Moat and Lichstein also found that adaptation of biotin-deficient cells of S. cerevisiae to sucrose fermentation was augmented in the presence of biotin which suggested to them that biotin was concerned with the synthesis of the adaptive enzyme. In 1958 Strauss and Moat reported that biotin stimulated glucose and fructose fermentation by yeast and that the effect was upon hexokinase activity since biotin stimulated fermentation of biotin-deficient yeast with glucose and fructose as substrates, but not with glucose-6-phosphate, fructose-6-phosphate, or

fructose-1,6-diphosphate. Also biotin did not stimulate phosphoglucose isomerase and glucose-6-phosphate dehydrogenase. Further, biotin stimulation was found using either dried cell suspensions or cell-free extracts although with the use of dried cell suspensions, a preincubation of the cells with biotin for a period of 30 to 40 minutes was needed. They attributed this delay to a permeability barrier which was eliminated upon disruption of the cells since the delay was not present in the cell free extracts. This also suggested a direct action by biotin upon hexokinase rather than upon synthesis of the enzyme.

Other workers have not been able to demonstrate a direct action of biotin on enzyme systems. Because biotin was found to be identical with Coenzyme R by West and Wilson (1939), it was immediately assumed that biotin functioned as a co-enzyme. Ochoa et al. (1947) found that decarboxylation of oxalacetate was reduced in liver slices from biotin-deficient turkeys. Although the enzyme could not be activated by biotin in vitro, biotin given to the living animal was effective. Other workers (Olson et al., 1948) found a reduced incorporation into carboxy-labeled succinate of $C^{14}O_2$ by cardiac muscle slices from biotin-deficient ducks. Again biotin added to the medium was without effect, but intraperitoneal injections of biotin restored enzymic activity to near normal values. It

thus appeared that biotin was involved in synthesis of the enzyme or its prosthetic group, but biotin was demonstrated not to be a part of the enzyme molecule.

Chang and Peterson (1951) suggested that certain bound forms of biotin, rather than the free acid, might be required for in vitro experiments. They were successful in concentrating soluble bound biotin in at least three components, but did not identify any of the components nor use them in any reactions. To follow the concentration of bound biotin the workers used L. arabinosus, which presumably requires free biotin, and L. casei which can utilize both free and some bound forms. Wright et al. (1951) identified one bound form of biotin, biocytin, as ϵ -N-biotinyl-L-lysine.

A recent report indicated a more direct relationship than synthesis of the enzyme between biotin and an enzyme. Lichstein (1957) has reported the presence of bound biotin in an oxaloacetic carboxylase preparation. A significant correlation was found between degree of purity of the enzyme and concentration of bound biotin present.

PROCEDURE

A series of six experiments was performed in the present study which had as its purposes the production of biotin deficiency in guinea pigs and the study of changes which occurred as a result of the deficiency. The first four experiments were used essentially to establish performance records for guinea pigs under conditions in this laboratory and to establish the method for producing the deficiency.

Different ways of comparing guinea pigs and groups of guinea pigs were used during the course of the study. A summary of kinds of comparisons is given below:

| | |
|---------------------------|---------------------------|
| Weight gain | Experiments I through VI |
| Food consumed | Experiments I through VI |
| Food efficiency | Experiments I through VI |
| Hemoglobin concentration | Experiments I through VI |
| Red blood cell volume | Experiments I through VI |
| Condition at autopsy | Experiments I through VI |
| Blood urea | Experiments II through VI |
| Blood nonprotein nitrogen | Experiments II through VI |
| Muscle biotin | Experiments II through IV |
| Hepatic biotin | Experiments II through VI |
| Hepatic nitrogen | Experiments II through VI |
| Hepatic moisture | Experiments V and VI |
| Hepatic fat | Experiments V and VI |
| Adrenal cholesterol | Experiment VI |
| Renal fat | Experiment VI |
| Organ size or weight | Variable |

To aid in identification of different experiments, diets, and guinea pigs, these three variables have been coded. A Roman numeral has been used to designate each of the six different experiments. The numeral is followed by the letter S if guinea pigs received a synthetic diet or the letter C if

guinea pigs received commercial rabbit chow. Since three different synthetic diets were used, the diets were designated S, S', and S"; S was the first, S', the second, and S", the third diet used. Additions made to the synthetic diet or the commercial rabbit chow were indicated by the first, or first and second, letter of the added compound. For example, since the synthetic diets did not contain biotin, the diets which did contain biotin were designated as diets SB, S'B, or S"B. To avoid confusion between groups receiving aureomycin or avidin, the group receiving aureomycin was identified by the letters Au and the groups receiving avidin were identified with the letter A. Individual guinea pigs in an experiment were identified by number. A summary of the code identification for the groups is given in Table 1.

Animals and Their Care

Animals

All guinea pigs used during the study were obtained from Gopher State Caviary, St. Paul, Minnesota. Guinea pigs were placed on a train in Minneapolis at 4:00 P.M. and arrived at the Railway Express office in Ames at 5:00 A.M. the following morning. Only male animals were ordered.

The ages of the animals varied from one experiment to another. At first older animals were thought more desirable because they would adjust more easily to a synthetic diet;

Table 1. Designation of experimental groups

| Experiment | Diet | Code designation of group |
|------------|---|---------------------------|
| I | Synthetic diet 1 + biotin | I SB |
| | Synthetic diet 1 + biotin, lettuce | I SBL |
| | Synthetic diet 1 + biotin, minerals | I SBM |
| | Synthetic diet 1 + biotin, minerals, aureomycin | I SBMAu |
| II | Synthetic diet 2 | II S' |
| | Synthetic diet 2 + biotin | II S'B |
| | Synthetic diet 2 + desthiobiotin | II S'D |
| | Synthetic diet 2 + sulfasuxidine | II S'S |
| III | Synthetic diet 3 | III S'' |
| | Synthetic diet 3 + biotin | III S''B |
| | Commercial rabbit chow | III C |
| IV | Synthetic diet 3 | IV S'' |
| | Synthetic diet 3 + avidin | IV S''A |
| | Synthetic diet 3 + biotin | IV S''B |
| | Synthetic diet 3 + biotin, avidin | IV S''BA |
| | Commercial rabbit chow | IV C |
| | Commercial rabbit chow + avidin | IV CA |
| V | Synthetic diet 3 + biotin | V S''B |
| | Synthetic diet 3 + avidin | V S''A |
| | Commercial rabbit chow | V C |
| | Commercial rabbit chow + avidin | V CA |
| VI | Synthetic diet 3 + biotin | VI S''B |
| | Synthetic diet 3 + avidin | VI S''A |

younger guinea pigs were obtained for the third, fourth, and sixth experiments because of the better possibility of depleting their biotin stores, but in Experiment V, older guinea pigs were used in hopes that a chronic biotin deficiency could be produced. Estimations of the beginning ages of the animals

for the experiments have been made and are summarized in Table 2. The estimations were based on beginning weights and weights at the end of the first week when the animals should have recovered from weight losses occurring during shipping.

Table 2. Estimations of group age at the beginning of experiments

| Group | Average beginning weight | Average weight at end of first week | Estimated group age |
|---------|--------------------------|-------------------------------------|---------------------|
| | (gm.) | (gm.) | (days) |
| I SB | 188 | 194 | |
| I SBL | 185 | 217 | |
| I SBU | 163 | 196 | 10 - 14 |
| I SBMAu | 162 | 183 | |
| II S' | 184 | 206 | |
| II S'B | 198 | 200 | |
| II S'D | 173 | 204 | 12 - 14 |
| II S'S | 221 | 218 | |
| III S"B | 110 | 150 | |
| III S" | 72 | 102 | 3 - 6 |
| III C | 107 | 128 | |
| IV S" | 90 | 102 | |
| IV S"B | 108 | 124 | |
| IV S"A | 135 | 175 | 4 - 7 |
| IV C | 111 | 124 | |
| V S"B | 182 | 214 | |
| V S"A | 181 | 219 | |
| V C | 190 | 234 | 12 - 15 |
| V CA | 204 | 243 | |
| VI S"B | 146 | 179 | |
| VI S"A | 148 | 158 | 6 - 8 |

Housing and care

For the first two experiments guinea pigs were housed individually in 8" x 10" x 8" cages with wire mesh bottoms suspended in a roll-away rack over wood shavings. For the last four experiments each guinea pig was housed in 18" x 12" x 8" wire mesh cage which was placed over a tray of wood shavings. The wood shavings were changed daily and the cages were changed and sterilized once a week. Water bottles were washed every two days, or oftener if necessary, and fresh distilled water was given daily.

For the very young guinea pigs small food dishes, in addition to metal ones already present, were placed in the cages during the first week of the experiment. Small containers of distilled water were also provided in addition to water bottles.

Food and water were supplied ad libitum at all times. Daily food intake records were kept for all animals. To prevent undue vitamin losses by long exposure to room temperature the approximate amount of food eaten within 24 hours, plus a slight excess, was placed in each food cup. In many cases this procedure made it necessary to collect scattered food pellets three or four times daily. Even so, guinea pigs sometimes would not have access to food when checked in the morning.

Guinea pigs removed from experiment

During the course of the experiments certain animals were eliminated for reasons not necessarily related to the dietary modifications. In three cases guinea pigs refused to eat the diet or ate such small quantities that they either died or became so weak they were sacrificed. Colds occurred and in five instances colds became so severe or lasted for such a long period of time that animals were removed from experiment. Three animals from control groups and one animal from an experimental group died from unexplained causes.

No guinea pigs were removed from the first experiment, but two were removed from the second. Guinea pig II S'D24 was a female and guinea pig II S'S15 died after 7 days on the experiment because he refused to eat.

Guinea pigs eliminated from the third experiment included three (III S"B4, III C7, and III S"14) removed because of colds, a fourth (III C6) which refused to eat, a fifth (III C10) which behaved very peculiarly, perhaps due to a middle ear infection, and a sixth (III S"B3) which was transferred to the experiment of another worker.

In Experiment IV, data from three guinea pigs could not be included in the results. Animal IV S"2 died after 18 days on the experiment, having had a cold most of that time. Guinea pig IV S"3 died after 6 days due mainly to poor food consumption, and guinea pig IV S"B6 died after 17 days on

experiment. It had developed the same peculiar behavior as guinea pig III C10.

Of 28 guinea pigs starting Experiment V, three were not suitable experimental animals. The first (V CA22) died 3 days after arrival; the second (V C18) died on the 11th day of the experiment after an illness of several days; the third (V S"B2) was sacrificed on the 41st day because a cold contracted during the second week of experiment had become increasingly severe.

Four guinea pigs were removed from the sixth experiment. Guinea pig VI S"B5 was a female; guinea pig VI S"A22 died on the fifth day due to a perforation of the lower bowel; guinea pig VI S"A19 was sacrificed after 15 days because it had had a severe cold for a week; and although guinea pig S"A23 completed the experiment, its data were not used because it refused to consume desired amounts of avidin.

All data from these guinea pigs have been eliminated from results discussed in this thesis.

Diets

Composition

Three synthetic diets were used in experiments reported here. The first diet, S, had been developed earlier by a worker in this laboratory and based on a diet satisfactory

for rats. Vitamins were included in amounts twice as large as those used by Woolley (1942) and Woolley and Sprince (1945).

After Reid and Briggs (1953) published their synthetic diet for guinea pigs, it was modified and used in the second experiment. The major differences between diet S' and the diet proposed by Reid and Briggs were in the type of bulk and in the carbohydrate source. Gum arabic was substituted for cellophane spangles and two carbohydrates, dextrin and glucose, were used instead of three, cornstarch, sucrose, and cerelese. The third synthetic diet, S'', was fed in Experiments III through VI and differed from the second, S', in that cellophane spangles and three carbohydrates, dextrin, sucrose, and glucose, were used. The compositions of the three synthetic diets are given in Table 3.

Approximately 0.25 mcg. of vitamin B₁₂ was fed per os daily to each guinea pig receiving a synthetic diet except in the final experiment when it was included as 0.4 gram B₁₂ with mannitol (0.1 per cent trituration) per kilogram of diet. When given orally B₁₂ was contained in one drop of a solution prepared by mixing 1 ml. of sterile saline containing 30 mcg. B₁₂ per ml. with 5 ml. of distilled water.

Ten milligrams of ascorbic acid in 0.5 ml. of a 40 per cent solution of glucose were given from a pipette each day to each guinea pig.

Table 3. Composition of the synthetic diets

| Ingredients | Diet | | |
|---------------------------------|-----------|----------------|-----------------|
| | S | S ⁱ | S ⁱⁱ |
| | (gm./kg.) | | |
| Vitamin-free casein | 300 | 300 | 300 |
| Gum arabic | 150 | 150 | - |
| Cellophane spangles | - | - | 150 |
| Salt mix ^a | 50 | 50 | 50 |
| Magnesium oxide | - | 5 | 5 |
| Potassium acetate | - | 25 | 23 |
| Swiftning | 20 | - | - |
| Corn oil ^b | 10 | 80 | 73 |
| Glucose | 325 | 255 | 161 |
| Sucrose | - | - | 117 |
| Dextrin | 140 | 130 | 116 |
| Choline chloride | 2 | 2 | 2 |
| Inositol | 2 | 2 | 2 |
| | (mg./kg.) | | |
| Thiamine HCl | 4.00 | 16.00 | 16.00 |
| Riboflavin | 10.00 | 16.00 | 16.00 |
| Pyridoxine HCl | 4.00 | 16.00 | 16.00 |
| Niacin | 20.00 | 200.00 | 200.00 |
| Calcium pantothenate | 20.00 | 40.00 | 40.00 |
| Folic acid | 0.12 | 10.00 | 10.00 |
| Para-aminobenzoic acid | 2.00 | - | - |
| 2-Methyl-1,4-naphthoquinone | 2.00 | 2.00 | 2.00 |
| Oleum percomorphum ^c | 300.00 | 600.00 | 300.00 |
| Wheat germ oil | 100.00 | - | - |
| Alpha-tocopherol acetate | - | 20.00 | 20.00 |

^aPhillips and Hart (1935)

^bMazola

^c300 mg. of oleum percomorphum contained approximately:
 18,000 U.S.P. units vitamin A
 2,550 U.S.P. units vitamin D

Guinea pigs which were fed commercial rabbit pellets received approximately 1250 U.S.P. units of vitamin A and 180 U.S.P. units of vitamin D once a week, provided by one drop of oleum percomorphum administered per os.

Preparation of diets

Vitamins were added to the synthetic diets as a mix consisting of thiamine, riboflavin, pyridoxine, calcium pantothenate, niacin, folic acid, para-aminobenzoic acid, 2-methyl-1,4-naphthoquinone, inositol, choline chloride, and glucose. The vitamins were ground together carefully with a mortar and pestle. Sufficient glucose was then added so that the vitamin mix could be incorporated as 5 per cent of the total diet. The vitamin mix was stored in green jars at -4° F. and used as needed in preparation of the diet.

Alpha-tocopherol acetate and oleum percomorphum were added to the diet by diluting first with the corn oil to be included in the diet.

Synthetic diet was prepared in a quantity sufficient for about 3 weeks. After mixing, the diet was pelleted for ease in keeping records of food intake and because it was believed that a pelleted diet would be more acceptable to guinea pigs. For the first two experiments the diet was mixed with distilled water (8 to 10 ml. per 100 grams of diet), rolled into a thin layer, cut into small pieces, and dried by air at 45° C.

or by infra red lamps at temperatures below 55° C.

Drying of the pellets for the first experiment required 4 to 5 hours at 45° C. For the second experiment the drying time was reduced to 2 to 3 hours by using infra red lamps under a hood. However, since this procedure required almost constant attention air-drying at 45° C. was adopted for the remaining experiments. When dry, the pellets were cooled and stored at -4° F. in tightly closed jars.

Isolation of avidin

Avidin was isolated according to the method developed by Dhyse (1954). Egg white, described as Egg-white Solid, Spray Type 12 (P-12), was obtained from Dr. Richard Forsythe of Henningsen, Inc., Springfield, Missouri.

Eight hundred grams of dried egg white were extracted at a time. This amount of dried egg white was dissolved in 8 liters of distilled water and the pH adjusted to 5.1 with 1 N hydrochloric acid. Nine liters of acetone were added slowly and the mixture stirred for 15 minutes. The mixture was filtered through Whatman No. 52 paper in Buchner funnels (24 cm. diameter). The precipitated egg white was suspended in 4 liters of distilled water, stirred 15 minutes, refiltered, suspended in 8 liters of 1 per cent sodium chloride, and stored overnight at 5° C. The saline filtrate obtained from this suspension was poured into lengths of cellophane tubing

and dialyzed for 2 days at approximately 5° C, the distilled water being changed three times daily. After the active fraction had precipitated, the tubes were emptied into large beakers, and rinsed with 1 per cent saline to dissolve the precipitate which clung to the sides. The rinsings were treated separately. Fifty grams of filter aid (Celite analytical filter aid, Johns-Mansville Co.) were added to the beaker containing the contents from the tubes minus saline rinsings. The Celite was filtered off, suspended in the saline rinsings, stirred for 30 minutes, and again filtered. When the saline solution was mixed with an equal volume of cold ethyl alcohol (-4° F.), avidin precipitated. The precipitate was centrifuged in a cold room (20° F.), washed with 50 per cent cold ethyl alcohol, recentrifuged, freeze-dried to a powder and stored at -4° F. The procedure was repeated until sufficient avidin was isolated for an experiment. Avidin was isolated at three different times from three batches of dried egg white, once each for Experiments IV, V, and VI.

Dhyse reported the purity of avidin prepared by this method as 20 per cent. By definition "pure" avidin contains 8 units per mg., one unit of avidin combining with 1 mcg. of biotin. In calculating the amounts of avidin to be fed, 20 per cent purity was assumed.

Experimental Plans

Experiments

Experiment I The first experiment was conducted for the purpose of testing a synthetic diet and the effect of aureomycin on guinea pigs receiving this synthetic diet. If aureomycin were non-toxic, it might be used as a bacteriostatic agent in subsequent experiments. Eight animals were divided randomly into four groups. All guinea pigs received biotin at the rate of 0.20 mg. per kilogram of ration. Biotin was the only addition to the first group, SB. In addition to biotin, the second group, SBL, received 15 grams of head lettuce daily; the third group, SBM, received 0.5 gram magnesium oxide and 2.3 grams potassium acetate per 100 grams of diet; and the fourth group, SBMAu, received the minerals and 11 mg. of aureomycin per 100 grams of diet.

Experiment II Three diets were used in Experiment II in an attempt to produce biotin deficiency. They were diet S', which contained no biotin and was a modification of that developed by Reid and Briggs (1953), diet S'S, which included the bacteriostatic agent, sulfasuxidine, and diet S'D, which included the analog desthiobiotin. A control group received diet S'B, which contained 0.60 mg. of biotin per kilogram of diet.

Desthiobiotin was added to the diet in amounts 5000 times that of the biotin fed to the control group, or 300 mg. dl-desthiobiotin per kilogram of diet. Sulfasuxidine was added as 1 per cent of the diet. The control group and the group receiving only the synthetic diet consisted of four guinea pigs each and the groups receiving the synthetic diet with desthiobiotin or sulfasuxidine had five guinea pigs each.

Experiment III Diet S" was used in the third experiment, a second attempt being made to produce a deficiency with a purified diet, but with younger guinea pigs than in Experiment II. Performance of control guinea pigs receiving synthetic diet plus biotin (S"B) was checked by a group fed commercial rabbit pellets (Purina Complete Rabbit Chow). The control group consisted of five animals receiving 0.60 mg. of biotin per kilogram of synthetic diet, the experimental group (S") consisted of four animals receiving only synthetic diet, and the third group (C) consisted of six animals receiving commercial rabbit pellets.

Experiment IV In Experiment IV, very young guinea pigs were fed synthetic diet S", and a few guinea pigs served in a pilot study for feeding avidin. Thirteen guinea pigs were divided into three groups, the three lightest being placed in the S" group. The beginning weights for the guinea pigs were 89, 91, 95, 109, and 135 grams for the group receiving diet S'; 102, 106, 106, and 109 grams for the group re-

ceiving diet S"B, which contained 0.60 mg. of biotin per kilogram of diet; and 107, 109, 118, and 118 grams for the group (C) receiving commercial rabbit pellets.

Avidin was fed to one guinea pig in each of the three diet groups. Guinea pigs receiving avidin were IV S"BA9, IV CA13, and IV S"A5. Starting on the 14th day, 1.5 mg. of the avidin preparation were given daily until the 35th day when the amount was raised to 2.0 mg. Each morning avidin was fed in 0.5 ml. of 0.1 per cent saline solution.

Experiment V The fifth experiment consisted of four diet groups. The first group received synthetic diet plus 0.60 mg. biotin per kilogram of diet; the second group received synthetic diet plus avidin; the third group received commercial rabbit pellets; and the fourth group received commercial rabbit pellets plus avidin. Avidin was withheld for the first 6 days of the experiment, then the S"A group received 1.5 mg. of the avidin preparation and the CA group received 3.0 mg. for 2 weeks. The amounts were doubled for both groups from the 21st day on. Avidin was fed in 0.5 ml. saline for the S"A group and 1.0 ml. for the CA group throughout the experiment, the entire amount being given in one feeding each morning.

Experiment VI The experiment consisted of two groups of 13 guinea pigs each. The first, or control group received synthetic diet with 0.60 mg. biotin added per kilogram of diet

and the second group received synthetic diet plus avidin. Avidin supplementation began the day the guinea pigs arrived. Six milligrams of the avidin preparation were given per day for the first 2 weeks, then the amount was raised to 18 mg. for the remainder of the experiment. Avidin was fed three times daily in a total of 1 ml. of 0.1 per cent saline solution. Guinea pigs received 0.3 ml. of the avidin solution in the morning and at noon and 0.4 ml. in late afternoon or early evening. Control guinea pigs received 1 ml. of saline each day.

Sacrifice and Autopsy of the Animals

Since tissues samples were required for analysis, guinea pigs were sacrificed when death seemed imminent and, if possible, a control guinea pig was sacrificed at the same time. The experiment was terminated and the remainder of the guinea pigs sacrificed after their period of rapid growth had passed. Guinea pigs which died were autopsied to ascertain possible causes of deaths and to check for organ changes, but tissues were not saved.

Guinea pigs were anesthetized by injecting 0.75 to 1.5 ml. pentobarbital sodium intraperitoneally. Each milliliter contained 0.45 grains pentobarbital sodium.

Blood was obtained by syringe from the portal vein or heart and emptied into vials containing dried double oxalate

(1.4 grams sodium oxalate and 0.8 grams potassium oxalate in 100 ml. distilled water, 0.1 ml. used per milliliter of blood). The contents of the vials were thoroughly mixed, the vials stoppered and refrigerated if the blood could not be measured immediately for the various determinations. Within 2 to 3 hours, determinations of hemoglobin and packed red blood cell volume were made and the protein-free filtrate of blood for urea and nonprotein nitrogen determinations were prepared.

Animals were exsanguinated by cutting the portal vein and organs removed for observation. Tissues to be analyzed were weighed, wrapped in aluminum foil, frozen, and stored at -4° F. Records were made of the gross appearance of the liver, kidneys, adrenal glands, spleen, reproductive organs, lungs, gastrointestinal tract, and fat stores. The gastrointestinal tract from a point immediately below the diaphragm to the anus was removed and weighed with contents.

Analyses

Hematology

Hemoglobin Hemoglobin as oxyhemoglobin was determined in triplicate by a modification of the procedure of Wintrobe (1946, p. 255). Twenty microliters of oxalated blood were measured into 8.0 ml. of freshly prepared 0.5 per cent ammonium hydroxide and the resulting color read in the Beckman DU quartz spectrophotometer at $540\text{ m}\mu$.

Packed red blood cell volume Packed red blood cell volumes were measured in triplicate with Van Allen hematocrit tubes. Blood was diluted with 1.3 per cent sodium oxalate and the filled tubes were centrifuged at 2500 r.p.m. for 45 minutes.

Urea and nonprotein nitrogen The blood filtrate used for urea determinations in Experiments II, III, and IV was prepared by tungstic acid precipitation according to Folin and Wu (1919). To 1 ml. of oxalated blood 7 ml. of distilled water were added, then 1 ml. of 10 per cent tungstate and 1 ml. of $2/3$ N sulfuric acid. In the fifth and sixth experiments when nonprotein nitrogen was also determined, modifications of this method were used. In the fifth experiment Haden's modification (1923) was used. Distilled water and $2/3$ N sulfuric acid were combined and added to the blood as 8 ml. of $1/12$ N sulfuric acid, in order to prevent loss of some nonprotein nitrogen precipitated by the original method. Van Slyke and Hawkin's modification (1928) was used in the sixth experiment. For precipitation of protein, all three reagents of the original procedure were mixed and added as a single reagent. Filtrates obtained by the above procedures were covered with toluene and stored in tightly stoppered test tubes at -4° F.

Colorimetric determinations of blood urea were made using the method of Archibald (1945). To 1 or 2 ml. of the 1:10

protein-free filtrate were added (1) water, (2) an acid mixture consisting of 1 volume concentrated sulfuric acid, 3 volumes syrupy phosphoric acid and 1 volume distilled water, and (3) a color reagent, α -isonitrosopropiophenone. Color was developed by boiling for 1 hour in a covered water bath with marbles being used to cover the test tubes rather than the cork-capillary stopper of the original method. Samples were then cooled quickly in a covered water bath at room temperature in a darkened room. The color was read in a Klett-Summerson photoelectric colorimeter using a No. 54 green filter.

One-milliliter aliquots of 1:10 protein-free blood filtrate were used for nonprotein-nitrogen determinations. Digestion (Wong's procedure, 1923) was carried out in 25 by 200 mm. tubes marked at 35 and 50 ml. with 1 ml. of 50 per cent sulfuric acid. After a 4-minute digestion period, 2 drops of hydrogen peroxide completed the clearing and the samples were digested for an additional minute, cooled, and diluted to 35 ml. with distilled water. Color was developed with Nessler's solution prepared according to Koch and McMeekin (1924). Nessler's solution was added 1 minute before reading since cloudiness usually developed within 4 to 5 minutes. A Klett-Summerson photoelectric colorimeter with a No. 54 green filter was used for the readings.

Adrenal cholesterol

For adrenal cholesterol determination the author developed a modification of the serum method published by Abell et al. (1952). The method published by Abell and coworkers required saponification of 0.5 ml. serum with 5 ml. alcoholic KOH, but 0.5 ml. of aqueous adrenal homogenate (total volume 25 ml.) contained too little cholesterol for color development. The present adaptation involved grinding adrenals in 2 ml. of absolute alcohol in a Potter-Elvehjem homogenizer, transferring to a 25 ml. volumetric flask and making to volume with absolute alcohol. Five milliliters of this homogenate were treated in the same manner as standards in the method of Abell and coworkers.

The procedure for determining cholesterol follows: 5 ml. of adrenal homogenate or standard cholesterol in absolute alcohol were measured into a 25 ml. glass-stoppered centrifuge tube, 0.3 ml. of 33 per cent aqueous KOH added, and the mixtures shaken. Saponification was accomplished by incubating the samples for 55 minutes at 38° C. in a water bath. After incubation, 10 ml. of petroleum ether were added to each tube and the samples shaken. Then 5 ml. of water were added, the samples again shaken, and centrifuged. An aliquot of the petroleum ether layer, which was transferred to a test tube, was evaporated in a water bath at 60° C. After the

samples were dried thoroughly, color was developed with a modified Liebermann-Burchard reagent (Abell et al., 1952) by incubating for 30 minutes at room temperature in a water bath which excluded light. Color was read in a Beckman Model B spectrophotometer at 620 $m\mu$.

Analyses of liver

Preparation of liver homogenate Livers were homogenized with acetate buffer (pH 4.6 - 4.8) in a Waring blender, transferred quantitatively to a volumetric flask and made to volume with distilled water. Since livers varied widely in weight, final volume was chosen so that a somewhat uniform concentration of tissue would be obtained. Buffer was used at a rate of 5 ml. per 25 ml. of final volume. Homogenates were covered with a layer of toluene and stored in dark bottles at -4° F. until all determinations had been finished. When homogenates were thawed for the various analyses they were reblended before samples were withdrawn.

Hepatic nitrogen Hepatic nitrogen was analyzed by the Kjeldahl-Gunning-Arnold procedure. Twenty milliliters of homogenate were digested with 25 ml. concentrated H_2SO_4 and a catalyst composed of 15 grams K_2SO_4 and 0.7 gram mercuric oxide. After reducing excess mercuric oxide with zinc dust and neutralizing the H_2SO_4 with saturated NaOH, ammonia was distilled into 2 per cent boric acid solution (modified from

Meeker and Wagner, 1933) and titrated with standard 0.1 N HCl using methylene blue-methyl red as an indicator.

Hepatic fat Hepatic fat was determined using the Soderhjelm and Soderhjelm (1949) method. Ten milliliters of liver homogenate were extracted twice with ethyl alcohol, ethyl ether, and petroleum ether. The first extraction was made with 15 ml. ethyl alcohol, 20 ml. ethyl ether and 25 ml. petroleum ether and the second with 5 ml., 15 ml., and 15 ml., respectively of the three solvents. The solvents were evaporated over a water bath and the fat dried for 14 hours at 50° C. in a vacuum oven.

Hepatic moisture Five milliliters of homogenate were dried to constant weight in small weighed aluminum cups in an air oven at 75° C.

Hepatic biotin Duplicate 1-ml. aliquots of liver homogenates were assayed microbiologically for biotin. The aliquots were autoclaved for 1 hour at 15 pounds pressure with 15 ml. of 3 N H₂SO₄. After the samples were cooled, they were transferred to volumetric flasks, diluted to approximately 3/4ths of their final volume, and their pH adjusted to 6.6 - 6.8 with 5 N NaOH using brom thymol blue as an external indicator. Then the samples were diluted to volume.

To correct for inhibiting effects of the Na₂SO₄ in the samples on growth of L. arabinosus, Na₂SO₄ was added to the

basal medium of the standard curve in amounts equivalent to that obtained in a tube containing 3 ml. of the test material.

The medium used for biotin assay was a modification of that of the American Association of Agricultural Chemists and United States Pharmacopeia (1945, p. 362) and the composition is given in Table 4.

Table 4. Composition of 100 ml. double strength medium

| | |
|----------------------------------|---|
| Hydrolyzed casein (vitamin-free) | 10.0 ml. |
| Glucose | 4.0 gm. |
| Sodium acetate | 4.0 gm. |
| L-asparagine | 50.0 mg. |
| L-cystine | 40.0 mg. |
| Glutamic acid | 40.0 mg. |
| DL-tryptophan | 20.0 mg. |
| Xanthine | 2.0 mg. |
| Adenine | 2.0 mg. |
| Guanine | 2.0 mg. |
| Uracil | 2.0 mg. |
| Pantothenic acid | 80.0 mcg. |
| Riboflavin | 80.0 mcg. |
| Thiamine | 40.0 mcg. |
| Niacin | 80.0 mcg. |
| Pyridoxine | 40.0 mcg. |
| Para-aminobenzoic acid | 40.0 mcg. |
| Folic acid | 4.0 mcg. |
| Salts A | 1.0 ml. |
| Salts B | 1.0 ml. |
| Salts A | 100 mg. K_2HPO_4 /ml. 100 mg. KH_2PO_4 /ml. |
| Salts B | 40.0 mg. $MgSO_4 \cdot 7H_2O$ /ml. 2.0 mg. $NaCl$ /ml. 2.0 mg. $FeSO_4 \cdot 7H_2O$ /ml. 2.0 mg. $MnSO_4 \cdot 4H_2O$ /ml. |

After incubation at 37° C. for 72 hours, the micro-organisms were destroyed by autoclaving for 5 minutes and lactic acid was titrated with 0.1 N NaOH with brom thymol blue as the indicator.

Muscle biotin

The gastrocnemius muscle tissue was homogenized in 5 ml. of acetate buffer (pH 4.6 - 4.8) using a Potter-Elvehjem homogenizer and diluted to 25 ml. One-milliliter aliquots were assayed with L. arabinosus following the same procedure as that used for hepatic tissue.

Renal fat

Kidneys were homogenized in distilled water using a Waring blender, transferred quantitatively to a 50 ml. volumetric flask and made to volume. Ten-milliliter aliquots were extracted according to Soderhjelm and Soderhjelm (1949).

RESULTS AND DISCUSSION

Evaluation of Physical Changes

Evaluation of changes during a deficiency are often based in part on the appearance of the animal. György (1954) has described varying degrees of biotin deficiency in the rat. The incipiently deficient rat exhibited cessation of growth, spectacled eyes, and beginning alopecia. A slightly to moderately deficient rat had a combination of the above symptoms coupled with more marked alopecia, closure of the eyes by exudate, and red, inflamed nose and snout. A moderately to severely deficient animal had developed cracks and sores on the skin along with encrustations, scab formation, and seborrheic deposits. A severely deficient rat was almost completely denuded, walked with the kangaroo gait, had sores and cracking of the skin, particularly of the folds under the legs, closure of the eyes, and marked weight loss.

Guinea pigs in the present experiment, however, defied classification by this method because the gross manifestations which they developed were neither as severe nor as varied as the symptoms described above. Symptoms such as spectacled eye and kangaroo gait did not occur. The guinea pigs developed symptoms that were, in fact, very close to those described by Lease et al. (1937), namely, inactivity, a hunched position, and dull, unkempt hair. Lease and coworkers felt such

nonspecific symptoms in the guinea pig were not due to biotin deficiency but to toxicity of egg white in the diet.

In the present experiments, a slight scaliness of the feet or ears appeared occasionally in deficient guinea pigs, but the condition never persisted or became severe. The hair often seemed thinner on the deficient guinea pigs than on their controls, but definite denuded areas were observed on only two animals. Both of these guinea pigs were receiving synthetic diet plus avidin in Experiment VI, and lost hair in the ventral region. Bristly fur also appeared on the outer legs of one guinea pig and its hair lost some color. Since a large number of the guinea pigs were albinos the possible effect of biotin deficiency on hair color could not be evaluated.

The onset of deficiency was possibly too sudden for characteristic symptoms to develop during the last experiment. The majority of guinea pigs were autopsied between the fourth and fifth week, while the guinea pig exhibiting loss of hair color did so only at the end of the sixth week. Generally hair began to fall out during the fourth week. Coots et al. (1959) noted hair loss after approximately 4 weeks, but the decoloration of the coat did not appear until the eighth week.

Animals developed an emaciated appearance which was particularly noticeable if they survived after weight loss began. The term "unthriftiness" used by Gram and Okey (1958)

to describe their biotin-deficient rats was a good adjective for the usual appearance of deficient guinea pigs. Most animals were sacrificed after weight loss began because their condition became precarious quickly. Of 10 guinea pigs receiving avidin in Experiment VI, two guinea pigs collapsed and were sacrificed immediately and a third died. Of these three, two had had only slight weight losses, 6 and 9 grams, and one had lost 36 grams. The only outward gross symptoms for these three were cottony fur and retarded growth.

Effect of Diets on Hepatic Concentration of Biotin

Several groups of workers have measured hepatic stores of biotin to evaluate the severity of the deficiency which they had produced. Biotin was determined in the livers of guinea pigs beginning with those of Experiment II. Values for individual animals are given in Table 5 and average values for groups in Tables 6 and 7.

Synthetic diet

When the results for all groups receiving synthetic diets were compared with results for their control groups receiving synthetic diet plus biotin, no consistent difference was apparent. Only in Experiment III were concentrations of biotin lower in the group receiving the synthetic diet than in their controls. Age at the beginning of the experiment did

Table 5. Concentration of biotin in livers of guinea pigs of Experiments II through VI

| Animal | Final weight (gm.) | No. of days on expt. | Liver weight (gm.) | Total nitrogen (mg.) | Total biotin | | |
|----------|--------------------|----------------------|--------------------|----------------------|--------------|------------------|--------------|
| | | | | | (mcg./liver) | (mcg./gm. liver) | (mcg./mg. N) |
| II S'B1 | 496 | 81 | 16.3 | 532 | 12.0 | 0.74 | 0.023 |
| II S'B5 | 292 | 44 | 12.6 | 690 | 11.9 | 0.94 | 0.032 |
| II S'B14 | 320 | 81 | 25.0 | 690 | 18.9 | 0.76 | 0.027 |
| II S'17 | 501 | 81 | 17.1 | 549 | 14.0 | 0.82 | 0.026 |
| II S'2 | 425 | 74 | 13.5 | 470 | 12.6 | 0.93 | 0.027 |
| II S'9 | 342 | 43 | 12.2 | 344 | 10.1 | 0.99 | 0.029 |
| II S'18 | 506 | 74 | 20.0 | 602 | 21.3 | 1.07 | 0.035 |
| II S'21 | 537 | 74 | 24.7 | 698 | 20.9 | 0.84 | 0.030 |
| II S'D3 | 562 | 62 | 18.2 | 660 | 14.3 | 0.78 | 0.022 |
| II S'D6 | 292 | 41 | - | - | - | - | - |
| II S'D10 | 449 | 62 | 15.2 | 519 | 11.2 | 0.74 | 0.022 |
| II S'D16 | 486 | 62 | 14.1 | 494 | 14.7 | 1.04 | 0.030 |
| II S'S4 | 400 | 67 | 12.3 | 439 | 9.3 | 0.76 | 0.021 |
| II S'S13 | 427 | 67 | 15.2 | 517 | 11.8 | 0.78 | 0.023 |
| II S'S22 | 437 | 67 | 15.3 | 521 | 15.5 | 1.01 | 0.030 |
| II S'S23 | 440 | 67 | 15.0 | 483 | 9.0 | 0.60 | 0.019 |

Table 5. (Continued)

| Animal | Final weight (gm.) | No. of days on expt. | Liver weight (gm.) | Total nitrogen (mg.) | Total biotin | | |
|-----------------------|--------------------|----------------------|--------------------|----------------------|--------------|------------------|--------------|
| | | | | | (mcg./liver) | (mcg./gm. liver) | (mcg./mg. N) |
| III S"B1 | 352 | 44 | 16.3 | 497 | 16.5 | 1.01 | 0.033 |
| III S"B2 | 423 | 44 | 16.5 | 545 | 15.8 | 0.96 | 0.029 |
| III S"B5 | 297 | 37 | 11.8 | 380 | 11.9 | 1.01 | 0.031 |
| III C8 | 352 | 33 | 16.1 | 513 | 14.7 | 0.92 | 0.029 |
| III C9 | 330 | 37 | 14.8 | 475 | 12.4 | 0.84 | 0.026 |
| III C11 | 293 | 38 | 16.0 | 518 | 15.9 | 0.99 | 0.030 |
| III S"12 | 407 | 44 | 22.2 | 640 | 17.6 | 0.79 | 0.028 |
| III S"13 | 258 | 41 | 11.6 | 277 | 6.5 | 0.56 | 0.024 |
| III S"15 | 97 | 19 | 3.5 | 120 | 11.8 | 0.52 | 0.015 |
| IV S"1 | 335 | 55 | 15.3 | 461 | 12.1 | 0.79 | 0.026 |
| IV S"4 | 110 | 30 | 4.2 | 139 | 3.2 | 0.76 | 0.023 |
| IV S"A5 ^a | 206 | 32 | 8.2 | 279 | 3.3 | 0.40 | 0.012 |
| IV S"B7 | 174 | 34 | 7.0 | 216 | 6.2 | 0.88 | 0.028 |
| IV S"BA9 ^b | 280 | 60 | 8.5 | 344 | 7.0 | 0.82 | 0.020 |

^aFed avidin 18 days

^bFed avidin 46 days

Table 5. (Continued)

| Animal | Final weight (gm.) | No. of days on expt. | Liver weight (gm.) | Total nitrogen (mg.) | Total biotin | | |
|----------------------|--------------------|----------------------|--------------------|----------------------|--------------|------------------|--------------|
| | | | | | (mcg./liver) | (mcg./gm. liver) | (mcg./mg. N) |
| IV C10 | 256 | 34 | 8.1 | 263 | 6.1 | 0.75 | 0.023 |
| IV C11 | 444 | 59 | 24.0 | 722 | 23.7 | 0.99 | 0.033 |
| IV C12 | 293 | 34 | 12.5 | 392 | 10.1 | 0.81 | 0.026 |
| IV CA13 ^b | 371 | 60 | 15.2 | 501 | 10.2 | 0.67 | 0.020 |
| V S"B1 | 328 | 29 | - | - | - | - | - |
| V S"B3 | 575 | 60 | 27.8 | 873 | 20.6 | 0.74 | 0.024 |
| V S"B4 | 572 | 60 | 30.0 | 919 | 17.6 | 0.60 | 0.020 |
| V S"B5 | 515 | 52 | 21.0 | 729 | 20.5 | 0.93 | 0.028 |
| V S"B6 | 569 | 60 | 20.1 | 683 | 19.2 | 0.96 | 0.028 |
| V S"B7 | 537 | 60 | 24.3 | 799 | 19.5 | 0.81 | 0.024 |
| V S"A8 | 390 | 60 | 19.3 | 508 | 7.4 | 0.38 | 0.014 |
| V S"A9 | 471 | 60 | 20.8 | 678 | 13.9 | 0.67 | 0.021 |
| V S"A10 | 620 | 60 | 29.2 | 916 | 18.6 | 0.64 | 0.020 |
| V S"A11 | 642 | 60 | 24.9 | 824 | 16.6 | 0.63 | 0.020 |
| V S"A12 | 584 | 52 | 23.8 | 777 | 19.6 | 0.82 | 0.025 |
| V S"A13 | 450 | 52 | 17.2 | 617 | 11.5 | 0.67 | 0.019 |
| V S"A14 | 346 | 34 | - | - | - | - | - |

Table 5. (Continued)

| Animal | Final weight (gm.) | No. of days on expt. | Liver weight (gm.) | Total nitrogen (mg.) | Total biotin | | |
|----------|--------------------|----------------------|--------------------|----------------------|--------------|------------------|--------------|
| | | | | | (mcg./liver) | (mcg./gm. liver) | (mcg./mg. N) |
| V C15 | 615 | 61 | 21.5 | 657 | 25.9 | 1.20 | 0.039 |
| V C16 | 636 | 61 | 25.9 | 713 | 19.3 | 0.74 | 0.027 |
| V C17 | 500 | 50 | 15.5 | 532 | 11.9 | 0.77 | 0.022 |
| V C19 | 596 | 61 | 21.6 | 715 | 31.1 | 1.44 | 0.044 |
| V C20 | 636 | 61 | 22.6 | 706 | 25.9 | 1.14 | 0.041 |
| V C21 | 672 | 61 | 26.8 | 841 | 17.7 | 0.66 | 0.021 |
| V CA23 | 579 | 51 | 22.7 | 630 | 13.4 | 0.59 | 0.021 |
| V CA24 | 628 | 51 | 22.6 | 666 | 38.2 | 1.69 | 0.057 |
| V CA25 | 525 | 51 | 20.0 | 672 | 18.9 | 0.94 | 0.028 |
| V CA26 | 586 | 51 | 18.3 | 577 | 18.6 | 0.74 | 0.032 |
| V CA27 | 587 | 51 | 16.2 | 591 | 20.9 | 1.29 | 0.035 |
| V CA28 | 515 | 51 | 17.6 | 550 | 12.3 | 0.70 | 0.022 |
| VI S"B1 | 281 | 46 | 16.2 | 489 | 8.0 | 0.48 | 0.016 |
| VI S"B2 | 245 | 35 | 14.1 | 433 | 9.7 | 0.69 | 0.022 |
| VI S"B3 | 429 | 50 | 26.2 | 728 | 15.1 | 0.60 | 0.021 |
| VI S"B4 | 253 | 25 | 11.5 | 396 | 7.7 | 0.67 | 0.020 |
| VI S"B6 | 374 | 47 | 13.8 | 457 | 9.2 | 0.67 | 0.020 |
| VI S"B7 | 246 | 23 | 10.0 | 347 | 7.5 | 0.75 | 0.022 |
| VI S"B8 | 279 | 32 | 13.2 | 477 | 7.2 | 0.54 | 0.015 |
| VI S"B9 | 359 | 46 | 18.2 | 577 | 10.8 | 0.59 | 0.019 |
| VI S"B10 | 240 | 33 | 11.6 | 413 | 6.4 | 0.56 | 0.016 |
| VI S"B11 | 382 | 35 | 15.8 | 510 | 7.9 | 0.50 | 0.016 |
| VI S"B12 | 295 | 35 | 15.4 | 492 | 8.2 | 0.53 | 0.017 |
| VI S"B13 | 316 | 47 | 16.5 | 483 | 7.4 | 0.45 | 0.015 |

Table 5. (Continued)

| Animal | Final weight (gm.) | No. of days on expt. | Liver weight (gm.) | Total nitrogen (mg.) | Total biotin | | |
|----------|--------------------|----------------------|--------------------|----------------------|--------------|------------------|--------------|
| | | | | | (mcg./liver) | (mcg./gm. liver) | (mcg./mg. N) |
| VI S"A14 | 242 | 46 | 10.6 | 347 | 1.7 | 0.16 | 0.005 |
| VI S"A15 | 185 | 33 | 10.7 | 320 | 2.2 | 0.21 | 0.007 |
| VI S"A16 | 176 | 23 | 6.5 | 231 | 2.1 | 0.32 | 0.009 |
| VI S"A17 | 179 | 35 | 7.9 | 272 | 1.8 | 0.22 | 0.007 |
| VI S"A18 | 281 | 49 | 11.3 | 389 | 2.5 | 0.22 | 0.006 |
| VI S"A21 | 194 | 32 | - | - | - | - | - |
| VI S"A22 | 169 | 31 | 5.4 | 200 | 0.7 | 0.14 | 0.004 |
| VI S"A24 | 143 | 20 | 7.2 | 336 | 1.2 | 0.16 | 0.004 |
| VI S"A25 | 200 | 33 | 8.3 | 245 | 1.4 | 0.16 | 0.006 |
| VI S"A26 | 159 | 32 | 6.4 | 296 | 1.3 | 0.20 | 0.004 |

Table 6. Average hepatic concentrations of biotin (mcg./gm.), and ranges, diet groups of Experiments II through VI

| Experiment | Group | | | | | |
|------------|-------------|-------------|-------------|------|-------------|------------|
| | SB | S | C | SBA | SA | CA |
| II | | | | | | |
| Average | 0.81 | 0.96 | | | | |
| Range | 0.76 - 0.94 | 0.84 - 1.0 | | | | |
| III | | | | | | |
| Average | 0.99 | 0.63 | 0.92 | | | |
| Range | 0.96 - 1.0 | 0.52 - 0.79 | 0.83 - 0.99 | | | |
| IV | | | | | | |
| Average | 0.88 | 0.77 | 0.85 | 0.82 | 0.40 | 0.67 |
| Range | | 0.76 - 0.79 | 0.75 - 0.99 | | | |
| V | | | | | | |
| Average | 0.81 | | 0.99 | | 0.63 | 0.99 |
| Range | 0.60 - 0.92 | | 0.66 - 1.4 | | 0.38 - 0.82 | 0.59 - 1.7 |
| VI | | | | | | |
| Average | 0.59 | | | | 0.20 | |
| Range | 0.45 - 0.72 | | | | 0.16 - 0.32 | |

Table 7. Average hepatic concentrations of biotin (mcg./mg. N), and ranges, for diet groups of Experiments II through VI

| Experiment | SB | S | C | SBA | SA | CA |
|------------|---------------|---------------|---------------|-------|-----------------|---------------|
| II | | | | | | |
| Average | 0.027 | 0.030 | | | | |
| Range | 0.023 - 0.032 | 0.027 - 0.035 | | | | |
| III | | | | | | |
| Average | 0.031 | 0.022 | 0.028 | | | |
| Range | 0.029 - 0.033 | 0.015 - 0.024 | 0.026 - 0.031 | | | |
| IV | | | | | | |
| Average | 0.028 | 0.025 | 0.027 | 0.020 | 0.012 | 0.020 |
| Range | | 0.023 - 0.026 | 0.023 - 0.033 | | | |
| V | | | | | | |
| Average | 0.025 | | 0.032 | | 0.020 | 0.033 |
| Range | 0.020 - 0.028 | | 0.021 - 0.044 | | 0.014 - 0.025 | 0.021 - 0.057 |
| VI | | | | | | |
| Average | 0.018 | | | | 0.006 | |
| Range | 0.015 - 0.022 | | | | 0.0043 - 0.0087 | |

not explain this finding since guinea pigs in Experiment III were similar in age to guinea pigs in Experiment IV although in both experiments the guinea pigs were younger than guinea pigs of Experiment II.

Wright and Welch (1944) found that hepatic biotin of rats fed a purified diet was lowered from 1.5 mcg. per gram of liver of their control rats of the same age fed a stock diet containing biotin to 0.72 - 1.1 mcg. in 6 - 8 weeks and to 0.31 - 0.44 mcg. at the end of 12 weeks. With this degree of biotin depletion, no gross signs of biotin deficiency appeared. In the groups receiving synthetic diet in Experiments II, III, and IV only two guinea pigs, III S"15 and IV S"4, developed symptoms which were observed later in certain guinea pigs receiving avidin. Guinea pig III S"15 had the lowest hepatic concentration of biotin when compared with others of the same experiment and had weighed less than the other animals at the beginning of the experiment. Guinea pig IV S"4 had a high concentration of hepatic biotin, and also had been small when placed on experiment. Both, however, had low total amounts of biotin in the liver because their livers were small in size.

Synthetic diet plus dl-desthiobiotin

Individual guinea pigs of the group receiving dl-desthiobiotin all had hepatic concentrations of biotin which could

be considered normal. They averaged 0.85 mcg. per gram of liver, a concentration similar to 0.81 mcg. for the group receiving synthetic diet plus biotin and to 0.96 mcg. for the group receiving synthetic diet with no added biotin.

Biotin concentrations in livers of guinea pigs receiving dl-desthiobiotin did not differ from those of guinea pigs receiving synthetic diet with or without added biotin despite the inclusion of 300 mg. dl-desthiobiotin per kilogram of diet (5000 times the amount of biotin added to the control diet). Whether biotin in the liver was solely from that synthesized in the gut by microorganisms or partially from converted dl-desthiobiotin is not known. The value, 0.85 mcg. per gram of liver, reported for the group represented biotin only since dl-desthiobiotin neither replaces biotin nor acts as an anti-biotin in the presence of an exogenous supply of biotin for Lactobacillus arabinosus, the organism used for assay (Lilly and Leonian, 1944).

Synthetic diet plus sulfasuxidine

Guinea pigs receiving sulfasuxidine as 1.0 per cent of a synthetic diet had hepatic concentrations of biotin equal to those of animals receiving synthetic diet plus biotin or synthetic diet without added biotin. Guinea pig II S'S23 had the lowest liver biotin of all animals in Experiment II, but it had no symptoms of biotin deficiency other than an

unkempt appearance.

While biotin deficiencies in rats have been reported when sulfasuxidine was fed as 0.5 or 1.0 per cent of the diet, hepatic biotin values have been reported only for experiments which included sulfasuxidine as 2.0 per cent of the diet. Wright and Welch (1944) found that hepatic biotin in rats fed a purified diet containing 2.0 per cent sulfasuxidine was lowered from 0.70 - 1.1 mcg. per gram of liver to 0.41 - 0.72 mcg. after 6 weeks, to 0.20 - 0.42 mcg. after 8 weeks, and to 0.18 - 0.32 after 12 weeks. On the basis of gross symptoms Wright and Welch concluded that deficiency became evident when hepatic biotin concentration fell below 0.35 mcg. per gram of liver.

Skegg and Wright (1946) have reported that inclusion of 2.0 per cent sulfasuxidine in highly purified diets produced a combined folic acid and biotin deficiency in rats. Rats developed alopecia, porphyrin-stained whiskers, sore eyes, and rough coats. When different carbohydrates were included in the diets the authors noted that 2.0 per cent sulfasuxidine was equally effective in producing a deficiency. One group of rats, that receiving dextrin and 2.0 per cent sulfasuxidine, had symptoms of biotin deficiency with 0.64 mcg. biotin per gram of liver. This concentration was considerably higher than the value which Wright and Welch (1944) had concluded was critical for rats, namely 0.35 mcg. per gram of

liver.

Synthetic diet plus avidin

When avidin was fed to guinea pigs receiving synthetic diet, hepatic biotin was lowered in some guinea pigs. Guinea pig IV S"A5 was fed a synthetic diet, S", for two weeks and then 1.5 mg. of avidin concentrate per day was added. Two and one-half weeks later the guinea pig was losing weight and had an unkempt, emaciated appearance. Hepatic biotin at the end of 2 1/2 weeks was 0.40 mcg. per gram of liver, a value considerably lower than that of animals not receiving avidin in the same experiment.

In Experiment V, group V S"A (receiving 1.5 mg. avidin concentrate per day for 2 weeks, 3.0 mg. thereafter) had an average hepatic concentration of biotin lower than that of group V S"B, the values being 0.63 mcg. and 0.81 mcg. of biotin per gram of liver, respectively. However, the individual values within the two groups overlapped considerably, the range for the control group being 0.60 to 0.96 mcg. per gram of liver and for the S"A group, 0.38 to 0.82 mcg. Only the guinea pig having 0.38 mcg. biotin per gram of liver and guinea pig S"A14, which died, were retarded in growth; no others in the group had observable symptoms of biotin deficiency.

In Experiment VI, the average biotin concentration in

the liver for the group receiving avidin (6.0 mg. avidin concentrate per day for two weeks, 18.0 mg. thereafter) was lower than that of the control group whether expressed as concentration per gram of liver or per milligram of hepatic nitrogen. Also, the range of biotin concentrations in the VI S"A group was well below that of the control group. Controls averaged 0.59 mcg. biotin per gram of liver with a range of 0.44 to 0.72 mcg. and experimental animals averaged 0.20 mcg. with a range of 0.16 to 0.32 mcg. Expressed as micrograms of biotin per milligram of hepatic nitrogen, the group receiving avidin averaged 0.006 compared with 0.018 for their control group. All guinea pigs receiving avidin showed signs of biotin deficiency such as weight loss and unkempt appearance.

The differences in hepatic biotin concentrations between the animals receiving avidin in Experiments V and VI might be due (1) to differences in body stores of biotin at the beginning of the experiment since guinea pigs in Experiment V were older than those in Experiment VI and (2) to variation in the purity of the avidin preparation since it was isolated at different times for each experiment.

Diets containing both biotin and avidin

Feeding avidin to guinea pigs receiving biotin either in a synthetic diet (IV S"BA) or in commercial rabbit pellets

(IV CA13 and group V CA) was ineffective in lowering biotin concentrations in the liver. The amount of biotin available to guinea pigs from dietary sources and intestinal synthesis was apparently in excess of that necessary to bind the avidin fed. The good performance of guinea pigs receiving both biotin and avidin indicated that avidin was not toxic to guinea pigs in these experiments.

Relationship of Hepatic Concentration of Biotin to Deficiency Symptoms

Although Wright and Welch (1944) had concluded that in rats biotin concentration must fall below 0.35 mcg. per gram of liver before symptoms of a deficiency would appear, later, Skegg and Wright (1946) associated a much higher value, 0.62, with deficiency in rats receiving dextrin and sulfasuxidine. Terroine (1956) found that severe biotin deficiencies lowered hepatic concentrations of biotin, on the average, from 0.93 mcg. per gram in control rats to 0.18 mcg. However, some variation occurred in severely deficient rats, with concentrations ranging from 0.10 to 0.26 mcg. per gram of liver.

Guinea pig III S¹⁵ which exhibited gross symptoms of biotin deficiency such as reduced weight gain and unkempt appearance had a biotin concentration of 0.52 mcg. per gram of liver or 0.015 mcg. per milligram of hepatic nitrogen. These values are as high as those for several control guinea pigs in Experiment VI. However, the guinea pig had grown

very little and its liver was so small that it contained only 1.82 mcg. biotin. Guinea pig V S"A8, whose only indications of deficiency were slow growth and occasional weight loss, had a biotin concentration of 0.38 mcg. per gram of liver or 0.014 mcg. per milligram of hepatic nitrogen. The first guinea pig to receive avidin (IV S"A5) had a concentration of 0.40 mcg. biotin per gram of liver or 0.012 mcg. per milligram of nitrogen. Its total hepatic biotin was 3.30 mcg. This guinea pig had lost considerable weight and was shabby and unkempt in appearance. Guinea pigs receiving avidin in Experiment VI had very low concentrations of hepatic biotin, ranging from 0.16 to 0.32 mcg. per gram or from 0.0004 to 0.009 mcg. per milligram nitrogen. Total hepatic biotin ranged from 0.73 to 2.52 mcg. Although all guinea pigs receiving avidin in Experiment VI had symptoms of biotin deficiency at autopsy, they did not all have the same visible degree of deficiency.

The rate with which deficiency symptoms appeared was not associated with the degree of depletion of hepatic biotin. Within the deficient group, guinea pig S"A16, which had the highest concentration of biotin in the liver was autopsied on the 23rd day of the experiment because its condition seemed precarious due to weight loss. The only changes in its appearance were ruffled fur and a general unthrifty appearance. Guinea pig S"A24, which was autopsied only 2 days

later because of a prolapsed anus, had a hepatic biotin concentration that was only one-half that of S"A16. Again the only change noted was in hair texture. The guinea pig with the lowest concentration of hepatic biotin (S"A22) collapsed during the 31st day, but other deficient guinea pigs (S"A15, 17, 25, and 26) autopsied within the next few days had values which were nearly average for this experimental group. All had the same gross symptoms of weight loss and cottony fur. Guinea pig S"A14, which was on experiment for 46 days, had one of the lowest concentrations, but guinea pig S"A18, which was on experiment for 49 days, had one of the highest concentrations. Hence, lower hepatic biotin concentrations in Experiment VI were not necessarily associated with longer times on experiment.

Since variations of concentrations within the S"A group were small and since, in many cases, the length of time on experiment was not very different, a closer relationship between these factors may exist than the results of this experiment have indicated. Such small variations made it difficult to relate biotin concentration to severity of the gross symptoms which also varied only slightly.

All guinea pigs with the characteristic symptoms of retarded growth, weight loss, low food efficiency and cottony fur had total liver values of 3.30 mcg. or less. The concentration of hepatic biotin associated with development of

moderate to severe biotin deficiencies in guinea pigs of Experiment VI was 0.32 mcg. per gram of liver.

Biotin concentration in muscle

Gastrocnemius muscles obtained from guinea pigs of Experiments II, III, and IV were analyzed for biotin. The results are recorded in Table 8. No relationship between the concentration of biotin in the muscle and the concentration of biotin in the liver was observed. In Experiment IV guinea pig S"A5, which had the lowest hepatic biotin concentration, 0.40 mcg. per gram of liver, had an average biotin concentration for muscle of 0.048 mcg. per gram while S"B7, which had one of the highest hepatic biotin concentrations, 0.88 mcg. per gram of liver, had the lowest muscle biotin concentration, 0.029 mcg. per gram.

Terroine (1956) found that the concentration of muscle biotin in rats receiving egg white was reduced to approximately one-fifth that of controls. The controls averaged 0.047 mcg. biotin per gram of muscle, the biotin-deficient guinea pigs, 0.01.

Unfortunately muscle biotin determinations were not continued after the fourth experiment and biotin deficiencies were produced only in the sixth experiment. Terroine's findings might have been confirmed in this final experiment.

Table 8. Concentration of biotin in gastrocnemius muscle of guinea pigs of Experiments II, III, and IV

| Animal | Wt. of muscle removed (gm.) | Total biotin (mcg.) | Wt. biotin per gm. muscle (mcg.) |
|----------|-----------------------------|---------------------|----------------------------------|
| II S'B1 | 2.1 | 0.136 | 0.065 |
| II S'B5 | - | - | - |
| II S'B14 | 1.3 | 0.062 | 0.045 |
| II S'B17 | 1.9 | 0.098 | 0.052 |
| II S'2 | 1.6 | 0.058 | 0.036 |
| II S'9 | - | - | - |
| II S'18 | 2.4 | 0.139 | 0.058 |
| II S'21 | 2.2 | 0.129 | 0.059 |
| II S'D2 | 2.7 | 0.144 | 0.053 |
| II S'D6 | - | - | - |
| II S'D10 | 2.2 | 0.134 | 0.061 |
| II S'D16 | 2.1 | 0.146 | 0.070 |
| II S'S4 | 1.8 | 0.107 | 0.061 |
| II S'S13 | 2.0 | 0.129 | 0.065 |
| II S'S22 | 1.7 | 0.100 | 0.059 |
| II S'S23 | 2.1 | 0.119 | 0.057 |
| III S"B1 | 1.23 | 0.060 | 0.049 |
| III S"B2 | 2.31 | 0.110 | 0.048 |
| III S"B5 | 1.28 | 0.060 | 0.046 |
| III C8 | 1.90 | 0.103 | 0.054 |
| III C9 | - | - | - |
| III C11 | 1.21 | 0.061 | 0.050 |
| III S"12 | 1.92 | 0.090 | 0.047 |
| III S"13 | 1.01 | 0.048 | 0.048 |
| III S"15 | 0.17 | 0.067 | 0.040 |
| IV S"1 | 1.13 | 0.049 | 0.043 |
| IV S"4 | 0.33 | 0.014 | 0.044 |
| IV S"A5 | 0.50 | 0.024 | 0.048 |

Table 8. (Continued)

| Animal | Wt. of muscle removed (gm.) | Total biotin (mcg.) | Wt. biotin per gm. muscle (mcg.) |
|----------|-----------------------------|---------------------|----------------------------------|
| IV S"B7 | 0.49 | 0.014 | 0.029 |
| IV S"B8 | - | - | - |
| IV S"BA9 | 1.48 | 0.070 | 0.047 |
| IV C10 | 0.89 | 0.048 | 0.054 |
| IV C11 | 1.82 | 0.099 | 0.054 |
| IV C12 | 1.00 | 0.049 | 0.041 |
| IV CA13 | 1.82 | 0.103 | 0.056 |

Weight Gain, Food Intake, Food Efficiency

The essentiality of a vitamin is often assessed by the effect of its absence on growth and survival. Failure to grow may result from either reduced food intake or reduced ability to utilize food. The latter may be indicated by a reduced food efficiency if food intake remains relatively high.

Daily weight and food intake records were kept for each guinea pig. From these records, weight change, food consumption and food efficiency (weight gained per gram of food eaten) were calculated for each week of each experiment and for the entire experiment. Only data for animals completing the entire week were used in tables that were compiled on the weekly basis.

Experiment I

The first experiment was a preliminary investigation of the effect of aureomycin on the growth of guinea pigs receiving a synthetic diet containing biotin. Roine (1952) had noted unexpected effects of aureomycin when he was investigating synthesis of vitamins in the intestine of the guinea pig. After 2 days on a diet containing aureomycin, guinea pigs began to lose weight. Six out of nine guinea pigs were dead within 10 days. The remaining animals gained weight after a period of time, but two of them had died by the end of the sixth week. In 1955 Roine proposed an "infection theory" to explain these results. This theory suggested that the effect was due to suppression of the flora ordinarily found in the gut, hence allowing other microorganisms unhealthful to the animal to flourish. In his review of antibiotics in nutrition, Jukes (1955) referred to a private communication from Hogan stating that he had included aureomycin in guinea pig feed since 1952, and that he credited this antibiotic for his colony's ability to resist infectious enteritis.

Toxic effects of aureomycin were not observed in two guinea pigs in Experiment I. The guinea pigs were receiving a slightly higher level of aureomycin, 110 mg. per kilogram of diet, than that used by Roine, 100 mg. per kilogram of diet (1952).

The two animals receiving aureomycin responded quite differently to this antibiotic. Guinea pig I SBMAu7 frequently ate small amounts of food but never appeared ill although it lost weight for the first 2 weeks, while guinea pig I SBMAu8 ate well and grew well. As a result, wide differences in weight gains, food intakes, and food efficiencies were observed (Table 9). Guinea pig I SBMAu8 had an average daily weight gain of 5.1 grams per day while guinea pig I SBMAu7 averaged 2.0 grams. These gains were the highest and lowest gains during the experiment. Hence, it can be concluded that aureomycin added to the diet did not affect growth.

Both guinea pigs receiving aureomycin had frequent periods of diarrhea and their general appearance suggested that if the aureomycin were to be used as a bacteriostatic agent for prevention of biotin synthesis, all symptoms could not be attributed to biotin deficiency. Guinea pigs receiving either synthetic diet plus biotin or synthetic diet plus biotin and lettuce or synthetic diet plus biotin and minerals had similar gains and food efficiencies.

Although the guinea pigs receiving diets with and without aureomycin survived and grew fairly well, the synthetic diet used in Experiment I was abandoned in favor of the diet developed by Reid and Briggs (1953), who had reported an average daily weight gain of 6.1 grams for 6 weeks; a value

Table 9. Summary of weight gain, food intake, and food efficiency for guinea pigs during Experiment I

| Animal | Beginning weight (gm.) | Final weight (gm.) | No. of days on expt. | Final wt. increase (gm.) | Av. wt. gain (gm./day) | Av. food intake (gm./day) | Food efficiency (gm.) |
|----------|------------------------|--------------------|----------------------|--------------------------|------------------------|---------------------------|-----------------------|
| I SB1 | 207 | 315 | 42 | 118 | 2.8 | 10.5 | 0.27 |
| I SB2 | 164 | 330 | 42 | 161 | 3.8 | 11.7 | 0.32 |
| I SBL3 | 214 | 420 | 42 | 206 | 4.9 | 13.6 | 0.36 |
| I SBL4 | 156 | 308 | 42 | 152 | 3.6 | 8.3 | 0.44 |
| I SBM5 | 153 | 307 | 43 | 154 | 3.6 | 11.5 | 0.31 |
| I SBM6 | 173 | 377 | 43 | 204 | 4.7 | 13.9 | 0.34 |
| I SBMAu7 | 157 | 241 | 43 | 84 | 2.0 | 7.8 | 0.25 |
| I SBMAu8 | 168 | 382 | 42 | 214 | 5.1 | 12.6 | 0.40 |

considerably higher than that obtained here for the same period of time.

Experiment II

In the second experiment an attempt was made to produce a biotin deficiency with a synthetic diet, with the same diet plus dl-desthiobiotin or plus sulfasuxidine.

During the first week weight gains varied widely within all groups except the II S'D group (Table 10). Wide variations were also noted in food acceptance. In the S'S group three of four guinea pigs lost weight during the first week and one continued to lose weight during the second week. From the third week on, however, growth was fairly regular in each group with occasional variation within the groups due to infections or temporary reductions in food intake.

The S'D group grew more rapidly from the first week on than the S'B and S' groups, while the S'S group grew less rapidly. By the end of 8 weeks, the weight of the S'D group averaged 91 grams heavier than that of the S'S group and 45 grams heavier than that of the S'B and S' groups. The weight of the S'D group had averaged from 11 to 48 grams lower than the other groups at the beginning of the experiment.

Diarrhea and respiratory infections, which resulted in weight losses for two guinea pigs (S'B1, S'B14), affect not only their individual growth and food efficiency records

Table 10. Summary of weekly weight gain, food intake, and food efficiency of guinea pigs of Experiment II

| Week of study | | II S'B | II S' | II S'D | II S'S |
|--|--|----------------|--------------|-------------|--------------|
| 1 | No. of animals | 4 | 4 | 4 | 4 |
| | Av. initial wt. (gm.) | 184 | 198 | 173 | 221 |
| | Range | 152 - 218 | 161 - 242 | 140 - 201 | 196 - 260 |
| | Av. wt. at end of week (gm.) | 206 | 200 | 204 | 217 |
| | Range | 198 - 212 | 184 - 218 | 158 - 234 | 194 - 234 |
| | Av. wt. gain (gm./day) | 4.7 | 0.2 | 4.5 | -0.4 |
| | Range | -0.9 - 8.6 | -5.6 - 4.9 | 2.6 - 5.7 | -4.3 - 5.4 |
| | Av. food intake (gm./day) | 7.5 | 8.4 | 9.2 | 6.4 |
| | Range | 5.4 - 10.8 | 3.7 - 11.1 | 7.6 - 9.7 | 3.9 - 10.1 |
| | Av. wt. gain per gram food eaten (gm.) | 0.37 | 0.12 | 0.48 | -0.22 |
| | Range | -0.16 - 0.80 | -0.15 - 0.49 | 0.34 - 0.59 | -0.75 - 0.54 |
| | 2 | No. of animals | 4 | 4 | 4 |
| Av. wt. at end of week (gm.) | | 253 | 238 | 247 | 236 |
| Range | | 232 - 264 | 228 - 250 | 209 - 276 | 199 - 263 |
| Av. wt. gain (gm./day) | | 6.7 | 5.6 | 6.0 | 3.0 |
| Range | | 4.3 - 7.4 | 2.1 - 7.9 | 3.6 - 7.3 | -0.6 - 7.1 |
| Av. food intake (gm./day) | | 13.3 | 10.9 | 11.7 | 9.1 |
| Range | | 11.6 - 15.4 | 8.0 - 12.7 | 10.3 - 12.9 | 6.4 - 11.1 |
| Av. wt. gain per gram food eaten (gm.) | | 0.45 | 0.48 | 0.52 | 0.25 |
| Range | | 0.22 - 0.68 | 0.32 - 0.67 | 0.31 - 0.59 | -0.07 - 0.64 |

Table 10. (Continued)

| Week of study | | II S'B | II S' | II S'D | II S'S |
|--|--|----------------|-------------|--------------|-------------|
| 3 | No. of animals | 4 | 4 | 4 | 4 |
| | Av. wt. at end of week (gm.) | 286 | 268 | 272 | 276 |
| | Range | 256 - 308 | 245 - 285 | 248 - 291 | 242 - 311 |
| | Av. wt. gain (gm./day) | 4.6 | 4.3 | 3.6 | 5.6 |
| | Range | 3.4 - 7.4 | 1.9 - 5.6 | 1.0 - 5.6 | 3.6 - 6.7 |
| | Av. food intake (gm./day) | 14.2 | 12.0 | 12.2 | 11.4 |
| | Range | 11.7 - 16.0 | 11.7 - 12.4 | 18.6 - 14.0 | 6.0 - 15.3 |
| | Av. wt. gain per gram food eaten (gm.) | 0.32 | 0.35 | 0.28 | 0.52 |
| | Range | 0.21 - 0.50 | 0.16 - 0.45 | -0.12 - 0.43 | 0.40 - 0.63 |
| | 4 | No. of animals | 4 | 4 | 4 |
| Av. wt. at end of week (gm.) | | 306 | 284 | 299 | 302 |
| Range | | 287 - 330 | 267 - 298 | 281 - 334 | 261 - 330 |
| Av. wt. gain (gm./day) | | 3.0 | 2.4 | 3.9 | 3.7 |
| Range | | 1.4 - 4.4 | 1.0 - 4.6 | -1.1 - 7.3 | 2.4 - 5.3 |
| Av. food intake (gm./day) | | 12.8 | 13.2 | 14.9 | 12.8 |
| Range | | 10.0 - 16.1 | 12.6 - 13.6 | 9.6 - 18.1 | 11.6 - 13.9 |
| Av. wt. gain per gram food eaten (gm.) | | 0.23 | 0.17 | 0.22 | 0.29 |
| Range | | 0.14 - 0.31 | 0.08 - 0.34 | -0.12 - 0.40 | 0.18 - 0.40 |

Table 10. (Continued)

| Week of study | II S'B | II S' | II S'D | II S'S |
|--|-------------|-------------|-------------|--------------|
| 5 | | | | |
| No. of animals | 4 | 4 | 4 | 4 |
| Av. wt. at end of week (gm.) | 315 | 318 | 332 | 312 |
| Range | 239 - 340 | 296 - 334 | 292 - 410 | 286 - 340 |
| Av. wt. gain (gm./day) | 1.2 | 4.8 | 4.8 | 1.5 |
| Range | 0.3 - 2.9 | 3.0 - 8.1 | 1.3 - 10.9 | -0.7 - 3.6 |
| Av. food intake (gm./day) | 15.9 | 13.6 | 15.4 | 12.6 |
| Range | 12.4 - 18.9 | 12.9 - 14.0 | 13.3 - 18.9 | 9.3 - 15.0 |
| Av. wt. gain per gram food eaten (gm.) | 0.05 | 0.35 | 0.28 | 0.10 |
| Range | 0.01 - 0.16 | 0.21 - 0.58 | 0.10 - 0.58 | -0.08 - 0.24 |
| 6 | | | | |
| No. of animals | 4 | 4 | 3 | 4 |
| Av. wt. at end of week (gm.) | 334 | 354 | 385 | 336 |
| Range | 292 - 363 | 335 - 379 | 358 - 447 | 313 - 367 |
| Av. wt. gain (gm./day) | 2.8 | 5.1 | 5.2 | 3.4 |
| Range | 0.4 - 6.4 | 3.3 - 6.4 | 2.6 - 6.9 | 1.1 - 6.4 |
| Av. food intake (gm./day) | 19.8 | 17.6 | 20.3 | 16.6 |
| Range | 15.6 - 23.4 | 16.6 - 19.1 | 18.3 - 22.6 | 13.1 - 19.6 |
| Av. wt. gain per gram food eaten (gm.) | 0.14 | 0.29 | 0.30 | 0.21 |
| Range | 0.07 - 0.28 | 0.20 - 0.37 | 0.23 - 0.34 | 0.06 - 0.36 |

Table 10. (Continued)

| Week of study | II S'B | II S' | II S'D | II S'S |
|--|-------------|-------------|-------------|--------------|
| 7 | | | | |
| No. of animals | 3 | 3 | 3 | 4 |
| Av. wt. at end of week (gm.) | 393 | 406 | 418 | 369 |
| Range | 352 - 424 | 393 - 432 | 377 - 487 | 344 - 420 |
| Av. wt. gain (gm./day) | 6.3 | 6.6 | 4.3 | 4.7 |
| Range | 2.9 - 8.7 | 4.9 - 8.9 | 2.6 - 5.7 | -0.6 - 7.6 |
| Av. food intake (gm./day) | 18.4 | 22.8 | 20.2 | 17.6 |
| Range | 13.7 - 23.3 | 19.9 - 27.6 | 17.1 - 22.1 | 15.9 - 22.6 |
| Av. wt. gain per gram food eaten (gm.) | 0.33 | 0.31 | 0.21 | 0.26 |
| Range | 0.21 - 0.41 | 0.24 - 0.40 | 0.15 - 0.26 | -0.04 - 0.41 |
| 8 | | | | |
| No. of animals | 3 | 3 | 3 | 4 |
| Av. wt. at end of week (gm.) | 432 | 432 | 477 | 386 |
| Range | 367 - 471 | 414 - 442 | 429 - 546 | 379 - 391 |
| Av. wt. gain (gm./day) | 3.7 | 3.6 | 8.2 | 2.4 |
| Range | 1.8 - 6.7 | 1.1 - 7.0 | 7.4 - 9.4 | -5.9 - 6.6 |
| Av. food intake (gm./day) | 21.0 | 19.5 | 24.8 | 19.7 |
| Range | 19.9 - 22.9 | 14.4 - 24.1 | 24.3 - 25.3 | 14.1 - 21.7 |
| Av. wt. gain per gram food eaten (gm.) | 0.17 | 0.18 | 0.33 | 0.08 |
| Range | 0.09 - 0.29 | 0.06 - 0.29 | 0.30 - 0.37 | -0.41 - 0.30 |

Table 10. (Continued)

| Week of study | | II S'B | II S' | II S'D | II S'S |
|---------------|--|-------------|--------------|--------|-------------|
| 9 | No. of animals | 3 | 3 | | 3 |
| | Av. wt. at end of week (gm.) | 438 | 441 | | 400 |
| | Range | 368 - 493 | 397 - 491 | | 391 - 412 |
| | Av. wt. gain (gm./day) | 2.9 | 1.3 | | 2.0 |
| | Range | 0.1 - 4.6 | -2.4 - 7.0 | | 0.1 - 4.1 |
| | Av. food intake (gm./day) | 21.0 | 18.4 | | 18.1 |
| | Range | 15.3 - 25.0 | 14.3 - 24.7 | | 13.4 - 22.7 |
| | Av. wt. gain per gram food eaten (gm.) | 0.11 | 0.02 | | 0.09 |
| | Range | 0.01 - 0.18 | -0.17 - 0.28 | | 0.01 - 0.25 |
| 10 | No. of animals | 3 | 3 | | |
| | Av. wt. at end of week (gm.) | 456 | 483 | | |
| | Range | 378 - 512 | 423 - 537 | | |
| | Av. wt. gain (gm./day) | 2.5 | 6.1 | | |
| | Range | 1.4 - 3.4 | 3.7 - 7.9 | | |
| | Av. food intake (gm./day) | 22.7 | 22.3 | | |
| | Range | 22.4 - 24.4 | 18.7 - 26.3 | | |
| | Av. wt. gain per gram food eaten (gm.) | 0.11 | 0.27 | | |
| | Range | 0.06 - 0.16 | 0.20 - 0.36 | | |

Table 10. (Continued)

| Week of study | | II S'B | II S' | II S'D | II S'S |
|---------------|--|--------------|-------|--------|--------|
| 11 | No. of animals | 3 | | | |
| | Av. wt. at end of week (gm.) | 449 | | | |
| | Range | 368 - 490 | | | |
| | Av. wt. gain (gm./day) | -0.9 | | | |
| | Range | -3.1 - 1.7 | | | |
| | Av. food intake (gm./day) | 19.8 | | | |
| | Range | 13.4 - 23.4 | | | |
| | Av. wt. gain per gram food eaten (gm.) | -0.22 | | | |
| | Range | -0.23 - 0.08 | | | |

(Table 11), but also the record of group S'B for the 10th and 11th weeks (Table 10). Guinea pig S'B1 lost 84 grams during its last 10 days.

Comparison of the weight gains of groups S'B and S' indicated that a dietary source of biotin was not needed for growth by guinea pigs. Food intakes and food efficiencies were unaffected also by the absence of dietary biotin. These results confirmed Reid's findings (1954) that young guinea pigs grew well without a dietary supply of biotin.

The S'D group grew well with the exception of guinea pig S'D6, which was autopsied on the 41st day because of a prolapsed anus. Thus dl-desthiobiotin did not affect growth in guinea pigs during 8 weeks in which it was fed in amounts 5000 times that of biotin in the control diet. This, plus unchanged concentrations of hepatic biotin indicated that dl-desthiobiotin was not acting as an analog.

Emerson (1945) and Rubin et al. (1945) reported that dl-desthiobiotin had low activity for rats previously made biotin-deficient by consuming diets containing egg albumin. The latter workers estimated that the activity was 0.1 to 0.01 per cent that of biotin for rats.

Dittmer et al. (1944) observed that dl-desthiobiotin disappeared from the medium in which yeast was incubated. Since it was replaced by an equivalent amount of a substance possessing growth promoting powers for Lactobacillus casei,

Table 11. Summary of weight gain, food intake, and food efficiency for guinea pigs during Experiment II

| Animal | Beginning weight (gm.) | Final weight (gm.) | No. of days on expt. | Final wt. increase (gm.) | Av. wt. gain (gm./day) | Av. food intake (gm./day) | Food efficiency (gm.) |
|----------|------------------------|--------------------|----------------------|--------------------------|------------------------|---------------------------|-----------------------|
| II S'B1 | 197 | 496 | 81 | 249 | 3.0 ^a | 17.3 | 0.18 |
| II S'B5 | 152 | 292 | 44 | 140 | 3.2 | 12.2 | 0.26 |
| II S'B14 | 218 | 320 | 81 | 102 | 1.3 ^b | 15.4 | 0.08 |
| II S'B17 | 169 | 501 | 81 | 332 | 4.1 | 17.8 | 0.23 |
| II S'2 | 188 | 425 | 74 | 237 | 3.2 | 14.7 | 0.22 |
| II S'9 | 161 | 342 | 43 ^c | 181 | 4.2 | 13.0 | 0.32 |
| II S'18 | 202 | 506 | 74 | 304 | 4.1 | 16.7 | 0.25 |
| II S'21 | 242 | 537 | 74 | 295 | 4.0 | 17.2 | 0.23 |
| II S'D3 | 201 | 562 | 62 | 361 | 5.8 | 17.7 | 0.33 |
| II S'D6 | 186 | 292 | 41 ^d | 106 | 2.6 | 13.0 | 0.20 |
| II S'D10 | 140 | 449 | 62 | 309 | 5.0 | 16.0 | 0.31 |
| II S'D16 | 165 | 486 | 62 | 321 | 5.2 | 17.2 | 0.30 |
| II S'S4 | 196 | 400 | 67 | 204 | 3.0 | 15.1 | 0.20 |
| II S'S13 | 208 | 427 | 67 | 219 | 3.3 | 14.7 | 0.22 |
| II S'S22 | 219 | 437 | 67 | 218 | 3.2 | 13.8 | 0.24 |
| II S'S23 | 206 | 440 | 67 | 234 | 3.5 | 12.7 | 0.28 |

^aDiarrhea and respiratory infection during last 8 days of experiment

^bDiarrhea and respiratory infection during last 10 days of experiment

^cAutopsied on the 43rd day because of an undetermined illness

^dAutopsied on the 41st day because of a prolapsed anus

they concluded that dl-desthiobiotin was transformed to biotin by yeast cells. Microflora present in the gut of the guinea pig may also be capable of performing this transformation. Although concentrations of biotin in the liver and muscle indicated that dl-desthiobiotin was not converted to biotin and stored, this does not rule out the possibility that some dl-desthiobiotin might have been converted to biotin either by microflora or by tissues of the guinea pig since the origin of biotin present in these tissues was not identified.

The amount of desthiobiotin fed might have been too low to act competitively with the biotin available to the guinea pig. For L. casei Rubin et al. (1945) determined that the molar inhibition ratio of dl-desthiobiotin was 17,000. In this experiment, 300 mg. dl-desthiobiotin was added per kilogram of diet, but since the amount of biotin synthesized in the gut and absorbed by the guinea pig was not known, the biotin-dl-desthiobiotin ratio could not be calculated.

The possibility that the experiment was not carried on for a sufficient length of time for dl-desthiobiotin to establish anti-biotin activity was unlikely since biotin stores in the liver were not diminished after 8 weeks.

Other than a slow initial adjustment to the diet and a lower food intake during the experiment, the group receiving sulfasuxidine did not vary greatly from the control group with respect to growth or food efficiency. On the basis of

biotin concentration of the liver, appearance, and growth, guinea pigs receiving the synthetic diet containing 1 per cent sulfasuxidine were not deficient. This finding is in contrast to the numerous reports that sulfasuxidine is effective in producing biotin deficiency in rats (Wright and Welch, 1944; Skegg and Wright, 1946; Daft et al., 1942; and Halevy et al., 1955).

Guinea pigs in the S'S group did gain most slowly in this experiment, but their lower food intakes may account for this result. These guinea pigs also were unkempt in appearance during the middle part of the experiment, suggesting biotin deficiency, but their appearance improved as the experiment progressed and their hepatic biotin concentrations were essentially unaffected.

The reports of results when diets containing sulfasuxidine were fed to rats do not usually include data on food consumption. Welch and Wright (1943) concluded that inclusion of sulfasuxidine in amounts up to 10 per cent of the ration had no known deleterious effects on rats when the remainder of the diet was a stock ration composed of relatively crude ingredients. Skegg and Wright (1946) did report that rats consumed less food when fed a diet containing 2.0 per cent sulfasuxidine, some rats refusing all food. When Woodruff et al. (1953) incorporated 1.0 per cent sulfasuxidine in the diet fed to guinea pigs symptoms resulted which responded,

in part, to folic acid. However acceptance of the diet was not discussed.

Experiment III

Guinea pigs, 2 to 4 days old, were chosen for Experiment III because they would have had less chance to store biotin than older animals. Such young animals might develop biotin deficiency on a purified diet which did not include chemicals to reduce microfloral growth in their intestines. Okey *et al.* (1951) had observed that a typical deficiency in rats was more difficult to produce if they were placed on diets when they weighed 55 grams instead of 45 grams.

Since the average growth rate in the two previous experiments had not approached 7 to 8 grams per day, the rate Cannon *et al.* (1946) considered normal for guinea pigs receiving a diet of natural foodstuffs, one group was fed commercial rabbit pellets to measure maximum weight gain in the laboratory environment. The commercial diet was readily available, consisted of natural foodstuffs, and was uniform enough in composition to serve for comparison of growth from one experiment to the next.

The weekly weight gains of the group receiving synthetic diet plus biotin equalled those of the group receiving commercial rabbit chow (Table 12). Variations within the two groups were also similar. Neither group gained 7 to 8 grams

Table 12. Summary of weekly weight gain, food intake, and food efficiency of guinea pigs of Experiment III

| Week of study | | III S ^B | III S ["] | III C |
|---------------|--|--------------------|--------------------|-------------|
| 1 | No. of animals | 3 | 3 | 3 |
| | Av. initial wt. (gm.) | 110 | 75 | 107 |
| | Range | 99 - 119 | 60 - 86 | 91 - 116 |
| | Av. wt. at end of week (gm.) | 150 | 102 | 128 |
| | Range | 132 - 168 | 70 - 125 | 114 - 142 |
| | Av. wt. gain (gm./day) | 5.8 | 3.9 | 3.0 |
| | Range | 4.7 - 7.0 | 1.4 - 5.6 | 2.1 - 3.7 |
| | Av. food intake (gm./day) | 8.0 | 5.7 | 7.2 |
| | Range | 6.9 - 8.9 | 3.9 - 7.1 | 5.7 - 8.6 |
| | Av. wt. gain per gram food eaten (gm.) | 0.74 | 0.64 | 0.42 |
| Range | 0.63 - 0.84 | 0.37 - 0.78 | 0.38 - 0.50 | |
| 2 | No. of animals | 3 | 3 | 3 |
| | Av. wt. at end of week (gm.) | 187 | 141 | 173 |
| | Range | 165 - 210 | 105 - 165 | 149 - 192 |
| | Av. wt. gain (gm./day) | 5.3 | 5.6 | 6.4 |
| | Range | 4.7 - 6.0 | 5.0 - 6.0 | 5.0 - 7.3 |
| | Av. food intake (gm./day) | 10.1 | 9.2 | 13.9 |
| | Range | 9.3 - 11.3 | 8.7 - 10.0 | 11.6 - 16.0 |
| | Av. wt. gain per gram food eaten (gm.) | 0.53 | 0.60 | 0.46 |
| | Range | 0.51 - 0.54 | 0.57 - 0.67 | 0.43 - 0.48 |

Table 12. (Continued)

| Week of study | | III S ["] B | III S ["] | III C |
|---------------|--|----------------------|--------------------|-------------|
| 3 | No. of animals | 3 | 2 | 3 |
| | Av. wt. at end of week (gm.) | 224 | 206 | 221 |
| | Range | 208 - 250 | 206 - 207 | 192 - 251 |
| | Av. wt. gain (gm./day) | 5.2 | 6.8 | 6.7 |
| | Range | 3.7 - 6.1 | 6.0 - 7.6 | 5.7 - 8.4 |
| | Av. food intake (gm./day) | 12.9 | 14.0 | 21.0 |
| | Range | 12.4 - 14.4 | 13.9 - 14.1 | 20.3 - 22.3 |
| | Av. wt. gain per gram food eaten (gm.) | 0.39 | 0.48 | 0.32 |
| | Range | 0.30 - 0.48 | 0.43 - 0.53 | 0.28 - 0.38 |
| 4 | No. of animals | 3 | 2 | 3 |
| | Av. wt. at end of week (gm.) | 270 | 256 | 270 |
| | Range | 245 - 311 | 242 - 269 | 233 - 317 |
| | Av. wt. gain (gm./day) | 6.6 | 7.0 | 7.0 |
| | Range | 4.8 - 8.7 | 5.0 - 9.0 | 5.6 - 9.4 |
| | Av. food intake (gm./day) | 16.0 | 17.8 | 20.1 |
| | Range | 14.4 - 17.7 | 16.6 - 19.0 | 17.7 - 25.6 |
| | Av. wt. gain per gram food eaten (gm.) | 0.40 | 0.39 | 0.34 |
| | Range | 0.31 - 0.47 | 0.30 - 0.47 | 0.32 - 0.37 |

Table 12. (Continued)

| Week of study | | III S ^B | III S ["] | III C |
|---------------|--|--------------------|--------------------|-------------|
| 5 | No. of animals | 3 | 2 | 2 |
| | Av. wt. at end of week (gm.) | 317 | 287 | 278 |
| | Range | 296 - 358 | 251 - 323 | 261 - 296 |
| | Av. wt. gain (gm./day) | 6.8 | 4.5 | 4.6 |
| | Range | 6.3 - 7.3 | 1.3 - 7.8 | 4.0 - 5.3 |
| | Av. food intake (gm./day) | 18.5 | 17.0 | 24.4 |
| | Range | 16.9 - 20.0 | 13.3 - 20.7 | 23.0 - 25.7 |
| | Av. wt. gain per gram food eaten (gm.) | 0.37 | 0.23 | 0.19 |
| Range | 0.34 - 0.43 | -0.10 - 0.37 | 0.17 - 0.21 | |
| 6 | No. of animals | 2 | 1 | |
| | Av. wt. at end of week (gm.) | 372 | 386 | |
| | Range | 338 - 405 | | |
| | Av. wt. gain (gm./day) | 6.2 | 9.0 | |
| | Range | 5.7 - 6.7 | | |
| | Av. food intake (gm./day) | 18.0 | 22.9 | |
| | Range | 17.7 - 18.3 | | |
| | Av. wt. gain per gram food eaten (gm.) | 0.34 | 0.39 | |
| Range | 0.32 - 0.37 | | | |

per day although one guinea pig on commercial rabbit pellets did average 7.2 grams (Table 13). Since guinea pigs were obtained from a supply house their growth potential was not known, and since guinea pigs vary genetically, a gain of 7 to 8 grams per day might not be achieved by all guinea pigs in any experiment.

The performance of guinea pigs in the S" group was quite variable. Guinea pig S"15 collapsed on the 19th day. It had been very small at the beginning of the experiment, but had gained weight and accepted the diet. Its low food efficiency, 0.28, and low average daily weight gain, 2.0 grams, for the experiment (Table 13) resulted from a decreasing food intake plus weight loss as the experiment progressed. Its hepatic biotin was lower than that of other guinea pigs in this experiment and its appearance was similar to those with biotin deficiencies in later experiments. Guinea pig S"13 also had a lower food efficiency and average daily weight gain for the experiment than any guinea pig receiving synthetic diet plus biotin. Its food efficiency and rate of weight gain diminished gradually as the experiment progressed, and it was sacrificed on the 42nd day after 2 weeks of fluctuating weight and inactivity. The third guinea pig in this group, S"12, had the best weight gain and food efficiency of all animals in the experiment.

The difference between the findings in this experiment

Table 13. Summary of weight gain, food intake, and food efficiency for guinea pigs during Experiment III

| Animal | Beginning weight (gm.) | Final weight (gm.) | No. of days on expt. | Final wt. increase (gm.) | Av. wt. gain (gm./day) | Av. food intake (gm./day) | Food efficiency (gm.) |
|----------|------------------------|--------------------|----------------------|--------------------------|------------------------|---------------------------|-----------------------|
| III S"B1 | 99 | 352 | 44 | 253 | 5.8 | 13.7 | 0.42 |
| III S"B2 | 119 | 423 | 44 | 304 | 6.9 | 15.4 | 0.45 |
| III S"B5 | 112 | 297 | 37 | 185 | 5.0 | 12.6 | 0.40 |
| III C8 | 116 | 352 | 33 | 236 | 7.2 | 18.4 | 0.39 |
| III C9 | 114 | 330 | 37 | 216 | 5.8 | 17.0 | 0.34 |
| III C11 | 91 | 293 | 38 | 202 | 5.3 | 16.5 | 0.32 |
| III S"12 | 78 | 407 | 44 | 329 | 7.5 | 15.8 | 0.48 |
| III S"13 | 86 | 258 | 41 | 172 | 4.2 | 12.5 | 0.34 |
| III S"15 | 60 | 97 | 19 | 37 | 2.0 | 7.2 | 0.28 |

and Experiment II may be due to the difference in initial weight of the animals since the rate of growth was the same in both experiments. The initial weight of the S' group in Experiment II averaged 198 grams compared with 75 grams for the S" group in Experiment III. Reid (1954) used guinea pigs with beginning weights ranging from 95 to 115 grams in the experiment from which she concluded that a dietary source of biotin was not necessary for guinea pigs. In Experiment III, weight gains and food efficiencies of three guinea pigs fed a synthetic diet lacking in biotin indicated that a dietary source of biotin may have been required by some of them.

Experiment IV

In the fourth experiment a second attempt was made to produce a biotin deficiency in young guinea pigs. In addition, an avidin preparation was given to three guinea pigs. Of these three, one received synthetic diet, one synthetic diet plus biotin, and one commercial rabbit pellets.

Many guinea pigs started poorly, with small food intakes and weight gains. Weekly weight gains, food intakes, and food efficiencies (Table 14) illustrate clearly the erratic behavior of all groups. Neither weight gains nor food efficiencies (Table 15) of control guinea pigs in the S"B and C groups were as good as in Experiment III. The last guinea pig remaining in the S"B group was autopsied during the fifth

Table 14. Summary of weekly weight gain, food intake, and food efficiency of guinea pigs during Experiment IV

| Week of study | Group | Initial weight (gm.) | Wt. at end of week (gm.) | Weight gain (gm./day) | Food intake (gm./day) | Wt. gain per gm. food eaten (gm.) |
|--------------------|-------------------------|----------------------|--------------------------|-----------------------|-----------------------|-----------------------------------|
| 1 | IV S"B (2) ^a | 108 | 124 | 2.4 | 5.8 | 0.30 |
| | Range | 106 - 109 | 106 - 142 | -0.4 - 5.1 | 3.6 - 7.1 | -0.12 - 0.72 |
| | IV S"BA ^b | 102 | 135 | 1.1 | 5.0 | 0.23 |
| | IV S" (2) | 90 | 102 | 1.6 | 6.8 | 0.23 |
| | Range | 89 - 91 | 96 - 107 | 0.7 - 2.6 | 3.6 - 9.9 | 0.20 - 0.26 |
| | IV S"Ab | 135 | 166 | 4.4 | 9.0 | 0.49 |
| | IV C (3) | 111 | 124 | 4.5 | 7.2 | 0.54 |
| | Range | 107 - 118 | 110 - 1137 | 2.1 - 7.6 | 4.9 - 10.3 | 0.34 - 0.74 |
| IV CA ^b | 118 | 173 | 7.9 | 12.0 | 0.66 | |
| 2 | IV S"B (2) | | 149 | 3.6 | 6.8 | 0.50 |
| | Range | | 120 - 178 | 2.0 - 3.7 | 6.9 - 11.0 | 0.42 - 0.58 |
| | IV S"BA ^b | | 152 | 6.0 | 8.1 | 0.74 |
| | IV S" (2) | | 134 | 4.6 | 6.8 | 0.73 |
| | Range | | 133 - 135 | 4.0 - 5.3 | 5.6 - 7.9 | 0.51 - 0.95 |
| | IV S"Ab | | 217 | 7.3 | 9.6 | 0.76 |
| | IV C (3) | | 184 | 6.2 | 13.0 | 0.47 |
| | Range | | 154 - 211 | 4.3 - 7.3 | 10.6 - 14.7 | 0.40 - 0.54 |
| IV CA ^b | | 207 | 5.0 | 12.5 | 0.38 | |

^aNumber of guinea pigs represented in average

^bAvidin not fed at this time

Table 14. (Continued)

| Week of study | Group | Initial weight (gm.) | Wt. at end of week (gm.) | Weight gain (gm./day) | Food intake (gm./day) | Wt. gain per gm. food eaten (gm.) |
|---------------|------------|----------------------|--------------------------|-----------------------|-----------------------|-----------------------------------|
| 3 | IV S"B (2) | | 166 | 3.2 | 9.0 | 0.37 |
| | Range | | 139 - 204 | 2.7 - 3.7 | 6.9 - 11.0 | 0.34 - 0.40 |
| | IV S"BA | | 178 | 3.7 | 11.7 | 0.32 |
| | IV S" (2) | | 162 | 4.0 | 8.0 | 0.48 |
| | Range | | 154 - 169 | 3.0 - 4.9 | 7.4 - 8.7 | 0.40 - 0.56 |
| | IV S"A | | 265 | 6.9 | 14.0 | 0.49 |
| | IV C (3) | | 227 | 6.2 | 19.8 | 0.31 |
| | Range | | 200 - 246 | 3.4 - 8.6 | 19.1 - 20.7 | 0.18 - 0.41 |
| | IV CA | | 235 | 3.9 | 16.3 | 0.24 |
| 4 | IV S"B (2) | | 181 | 1.4 | 11.2 | 0.11 |
| | Range | | 140 - 222 | 0.1 - 2.6 | 10.3 - 12.0 | 0.01 - 0.21 |
| | IV S"BA | | 217 | 5.6 | 12.0 | 0.46 |
| | IV S" (2) | | 168 | 0.8 | 9.2 | -0.22 |
| | Range | | 132 - 203 | -5.3 - 7.0 | 5.4 - 13.1 | -0.97 - 0.53 |
| | IV S"A | | 251 | -2.0 | 9.4 | -0.21 |
| | IV C (3) | | 276 | 6.9 | 23.7 | 0.29 |
| | Range | | 254 - 300 | 5.4 - 7.7 | 22.7 - 24.9 | 0.23 - 0.34 |
| | IV CA | | 238 | 0.4 | 19.1 | 0.02 |
| 5 | IV S"BA | | 217 | 4.6 | 8.6 | 0.53 |
| | IV S" | | 247 | 6.3 | 13.9 | 0.45 |
| | IV C | | 309 | 5.1 | 23.9 | 0.22 |
| | IV CA | | 244 | 0.9 | 16.0 | 0.05 |

Table 14. (Continued)

| Week of study | Group | Initial weight (gm.) | Wt. at end of week (gm.) | Weight gain (gm./day) | Food intake (gm./day) | Wt. gain per gm. food eaten (gm.) |
|---------------|---------|----------------------|--------------------------|-----------------------|-----------------------|-----------------------------------|
| 6 | IV S"BA | | 217 | 4.6 | 8.6 | 0.53 |
| | IV S" | | 275 | 4.0 | 14.1 | 0.28 |
| | IV C | | 347 | 5.4 | 31.0 | 0.18 |
| | IV CA | | 290 | 6.6 | 17.4 | 0.38 |
| 7 | IV S"BA | | 250 | 4.7 | 9.0 | 0.52 |
| | IV S" | | 317 | 6.0 | 15.0 | 0.40 |
| | IV C | | 390 | 6.1 | 39.0 | 0.19 |
| | IV CA | | 324 | 4.9 | 20.4 | 0.24 |
| 8 | IV S"BA | | 268 | 2.3 | 13.3 | 0.19 |
| | IV C | | 434 | 6.3 | 31.7 | 0.20 |
| | IV CA | | 353 | 4.1 | 20.9 | 0.20 |

Table 15. Summary of weight gain, food intake, and food efficiency for guinea pigs during Experiment IV

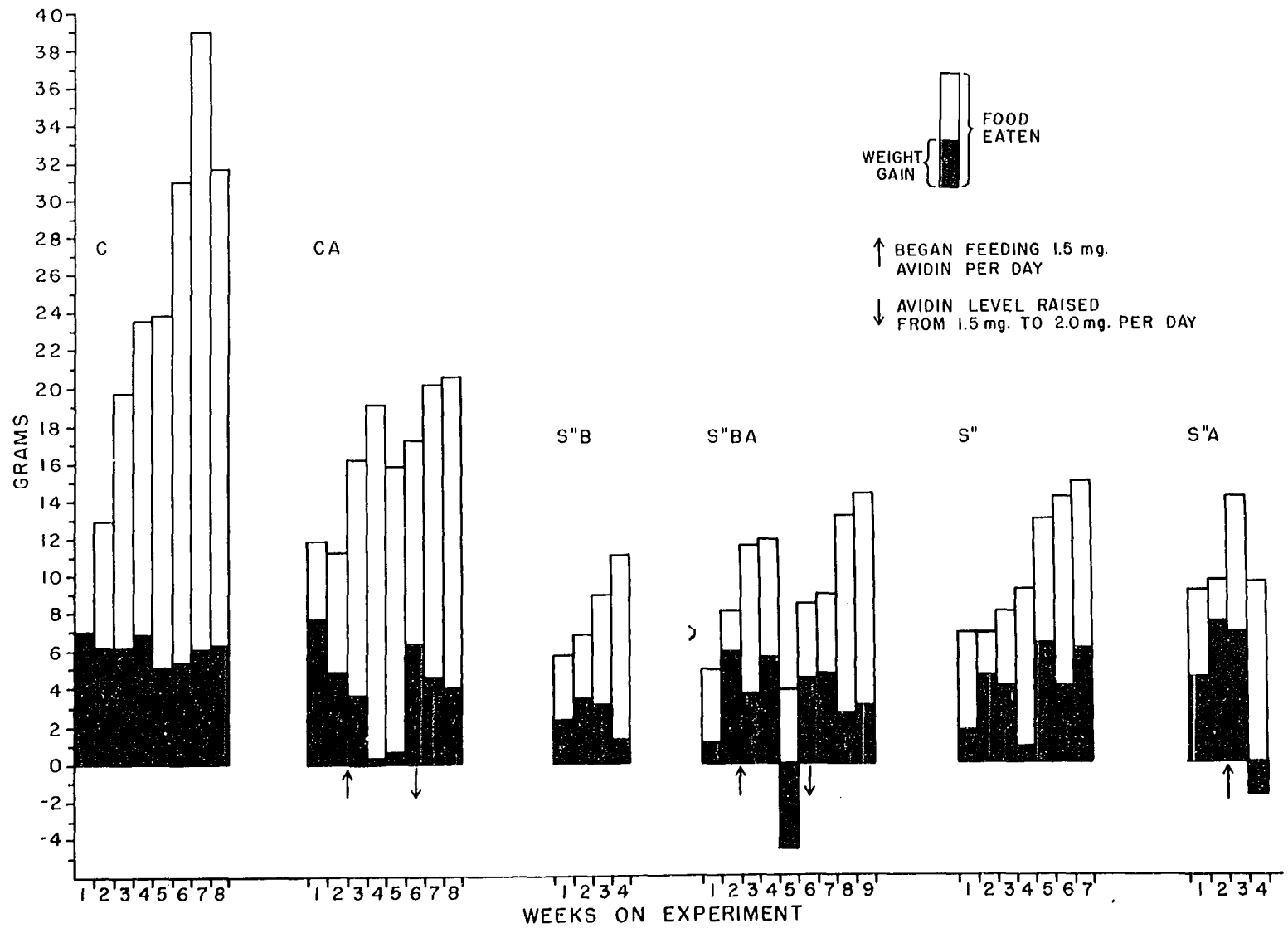
| Animal | Beginning weight (gm.) | Final weight (gm.) | No. of days on expt. | Final wt. increase (gm.) | Av. wt. gain (gm./day) | Av. food intake (gm./day) | Food efficiency (gm.) |
|----------|------------------------|--------------------|----------------------|--------------------------|------------------------|---------------------------|-----------------------|
| IV S"1 | 91 | 335 | 55 | 244 | 4.4 | 10.7 | 0.41 |
| IV S"4 | 89 | 110 | 30 | 21 | .7 | 7.6 | 0.09 |
| IV S"A5 | 135 | 206 | 32 | 71 | 2.2 | 10.0 | 0.22 |
| IV S"B7 | 109 | 174 | 34 | 65 | 1.9 | 6.6 | 0.29 |
| IV S"B8 | 106 | 218 | 29 | 112 | 3.9 | 9.7 | 0.40 |
| IV S"BA9 | 102 | 280 | 60 | 178 | 3.0 | 9.3 | 0.32 |
| IV C10 | 118 | 256 | 34 | 138 | 4.1 | 16.2 | 0.27 |
| IV C11 | 107 | 444 | 59 | 337 | 5.7 | 24.8 | 0.23 |
| IV C12 | 109 | 293 | 34 | 184 | 5.4 | 16.5 | 0.33 |
| IV CA13 | 118 | 371 | 60 | 253 | 4.2 | 16.9 | 0.25 |

week as the control for guinea pig S"A5, thus the weekly records for this group, Table 14 and Figure 1, are for 4 weeks only.

Guinea pigs S"1 and S"4 both had weight gains similar to those of guinea pigs in the S"B group for the first 3 weeks. Guinea pig S"1 continued to grow fairly well throughout the experiment but the condition of S"4 deteriorated during the fourth week, when it lost an average of 5.3 grams per day. Although 100 mcg. of biotin was given on the 25th day and 50 mcg. the following day, guinea pig S"4 continued to fail. The biotin may have been administered after irreversible changes had occurred or weight loss might have been due to something other than biotin deficiency. This animal did have a high hepatic biotin, 0.76 mcg. per gram, perhaps due to the large doses of biotin administered on the 2 days prior to autopsy.

Of the three guinea pigs fed avidin, only the guinea pig receiving the synthetic diet without biotin showed definite changes. The guinea pigs were not fed avidin during the first 2 weeks of the experiment. During these 2 weeks, their weight gains, food intakes and food efficiencies were comparable to other guinea pigs in the experiment. During the first week that guinea pig IV S"A5 received avidin it maintained a good food intake, weight gain and food efficiency record, but during the second week began to lose weight with only a small

Figure 1. Average daily weight gains and food intakes of diet groups of Experiment IV



reduction in food intake. Weight loss continued until it was sacrificed on the 19th day after avidin feeding began. Guinea pigs IV S"BA and IV CA both had periods of reduced growth (Tables 14 and 15 and Figure 1) during the time avidin was fed and by the end of the 8th week their weights were considerably smaller than the remaining control guinea pig.

Guinea pig IV CA ate less food than guinea pigs receiving only commercial rabbit pellets. Its food intake decreased after 1 week of avidin feeding. Guinea pig IV S"BA also reduced its food intake, beginning about 2 weeks after avidin feeding was initiated.

Of the three guinea pigs receiving avidin, only guinea pig S"A5 exhibited a reduction in hepatic biotin. It had the lowest hepatic biotin concentration, 0.40 mcg. per gram, of all animals in Experiments II, III, and IV. The biotin concentrations in the livers of guinea pigs IV S"BA and IV CA were similar to their controls.

The use of an avidin preparation appeared to be a satisfactory means for producing biotin deficiency in one guinea pig. By using isolated avidin rather than dried egg white, toxic reactions reported by Lease et al. (1937) were avoided. Further, rapid weight loss had not occurred when avidin was fed, so adjustment to its intake was not a problem.

Experiment V

Avidin was given to two groups of guinea pigs in this experiment. One group was receiving synthetic diet, the other, commercial rabbit chow. Somewhat larger and older guinea pigs, 140 to 234 grams, were used than in the two preceding experiments.

By studying the weekly summary table (Table 16), it was apparent that the average weight of the V S"A group fell progressively below the average weight of the V S"B group, the difference being 50 grams after 8 weeks. More important perhaps were the ranges of these two groups. Whereas the range of weights within the S"B group remained at approximately 60 grams, in the S"A group the range increased from 38 to 160 grams as the experiment progressed. Differences within the experimental group can be seen in the graphs of their individual average daily weight gains and food intakes (Figure 2). The amount of avidin preparation fed, 1.5 mg. daily for the first 2 weeks and 3.0 mg. daily for the remainder of the experiment, appeared to be producing a mild deficiency in some guinea pigs, but not in others.

Guinea pigs in Experiment V were older and larger than guinea pig IV S"A5, which developed a pronounced deficiency. Okey et al. (1951) found that it was more difficult to produce typical biotin deficiency syndromes in rats started at 45 grams compared to 55 grams. Many reports have stated that

Table 16. Summary of weekly weight gain, food intake, and food efficiency of guinea pigs during Experiment V

| Week of study | | V S"B | V S"A | V C | V CA |
|--|--|----------------|-------------|-------------|-------------|
| 1 | No. of animals | 6 | 7 | 6 | 6 |
| | Av. initial wt. (gm.) | 182 | 181 | 190 | 204 |
| | Range | 140 - 205 | 154 - 192 | 157 - 219 | 176 - 234 |
| | Av. wt. at end of week (gm.) | 214 | 219 | 234 | 243 |
| | Range | 177 - 235 | 170 - 261 | 195 - 276 | 211 - 270 |
| | Av. wt. gain (gm./day) | 4.5 | 5.5 | 6.4 | 5.6 |
| | Range | 1.4 - 6.9 | 1.6 - 7.4 | 5.3 - 8.4 | 1.9 - 8.3 |
| | Av. food intake (gm./day) | 11.5 | 11.8 | 19.4 | 19.1 |
| | Range | 9.9 - 12.4 | 8.6 - 16.7 | 11.9 - 25.4 | 17.0 - 24.3 |
| | Av. wt. gain per gram food eaten (gm.) | 0.40 | 0.46 | 0.34 | 0.29 |
| | Range | 0.12 - 0.56 | 0.18 - 0.61 | 0.26 - 0.46 | 0.11 - 0.42 |
| | 2 | No. of animals | 6 | 7 | 6 |
| Av. wt. at end of week (gm.) | | 253 | 259 | 279 | 292 |
| Range | | 220 - 295 | 194 - 307 | 249 - 345 | 212 - 325 |
| Av. wt. gain (gm./day) | | 5.6 | 5.9 | 6.4 | 8.0 |
| Range | | -2.1 - 9.6 | 3.4 - 11.3 | 4.4 - 9.8 | 6.0 - 10.1 |
| Av. food intake (gm./day) | | 13.0 | 13.4 | 22.9 | 23.6 |
| Range | | 6.6 - 15.9 | 9.3 - 17.1 | 19.8 - 29.3 | 21.3 - 27.1 |
| Av. wt. gain per gram food eaten (gm.) | | 0.36 | 0.41 | 0.28 | 0.34 |
| Range | | -0.33 - 0.60 | 0.26 - 0.66 | 0.22 - 0.36 | 0.27 - 0.48 |

Table 16. (Continued)

| Week of study | | V S "B | V S "A | V C | V CA |
|---------------|--|-------------|-------------|-------------|-------------|
| 3 | No. of animals | 6 | 7 | 6 | 6 |
| | Av. wt. at end of week (gm.) | 305 | 299 | 327 | 349 |
| | Range | 261 - 345 | 229 - 363 | 300 - 387 | 304 - 380 |
| | Av. wt. gain (gm./day) | 7.4 | 5.6 | 6.8 | 7.1 |
| | Range | 5.9 - 8.9 | 1.9 - 8.1 | 6.0 - 7.9 | 3.1 - 9.6 |
| | Av. food intake (gm./day) | 15.6 | 15.8 | 24.0 | 24.9 |
| | Range | 13.3 - 17.1 | 10.7 - 19.4 | 20.1 - 26.9 | 21.0 - 27.0 |
| | Av. wt. gain per gram food eaten (gm.) | 0.48 | 0.37 | 0.29 | 0.28 |
| | Range | 0.39 - 0.54 | 0.14 - 0.50 | 0.24 - 0.32 | 0.14 - 0.36 |
| 4 | No. of animals | 6 | 7 | 6 | 6 |
| | Av. wt. at end of week (gm.) | 349 | 343 | 361 | 398 |
| | Range | 311 - 379 | 282 - 424 | 238 - 430 | 332 - 421 |
| | Av. wt. gain (gm./day) | 6.2 | 6.3 | 7.3 | 7.1 |
| | Range | 2.1 - 8.0 | 2.6 - 8.7 | 6.1 - 8.6 | 4.0 - 9.4 |
| | Av. food intake (gm./day) | 18.4 | 16.9 | 28.6 | 27.5 |
| | Range | 17.3 - 21.0 | 13.4 - 20.9 | 24.7 - 31.3 | 22.9 - 31.4 |
| | Av. wt. gain per gram food eaten (gm.) | 0.34 | 0.36 | 0.26 | 0.26 |
| | Range | 0.12 - 0.46 | 0.28 - 0.50 | 0.20 - 0.30 | 0.14 - 0.35 |

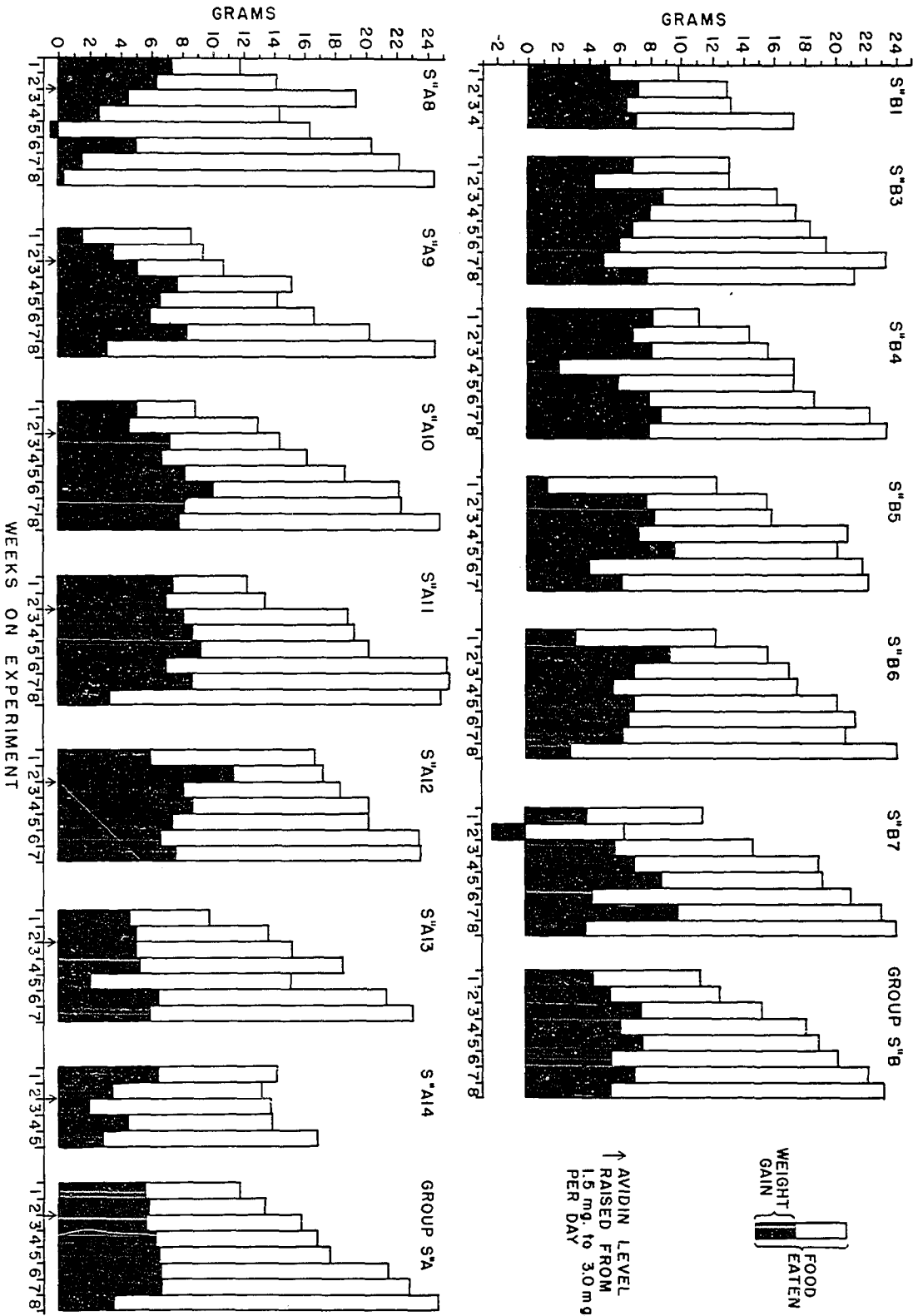
Table 16. (Continued)

| Week of study | | V S"B | V S"A | V C | V CA |
|--|--|----------------|--------------|-------------|-------------|
| 5 | No. of animals | 5 | 6 | 6 | 6 |
| | Av. wt. at end of week (gm.) | 409 | 384 | 438 | 459 |
| | Range | 372 - 436 | 294 - 475 | 420 - 507 | 404 - 493 |
| | Av. wt. gain (gm./day) | 7.7 | 6.6 | 8.6 | 8.7 |
| | Range | 6.0 - 9.7 | -0.6 - 9.3 | 5.9 - 11.0 | 5.6 - 10.3 |
| | Av. food intake (gm./day) | 19.0 | 17.6 | 31.5 | 32.0 |
| | Range | 16.9 - 20.4 | 15.1 - 20.6 | 25.3 - 37.1 | 27.7 - 26.1 |
| | Av. wt. gain per gram good eaten (gm.) | 0.40 | 0.30 | 0.27 | 0.32 |
| | Range | 0.35 - 0.48 | -0.04 - 0.45 | 0.19 - 0.32 | 0.24 - 0.50 |
| | 6 | No. of animals | 5 | 6 | 6 |
| Av. wt. at end of week (gm.) | | 450 | 430 | 489 | 514 |
| Range | | 405 - 484 | 329 - 524 | 445 - 536 | 462 - 547 |
| Av. wt. gain (gm./day) | | 5.9 | 6.6 | 7.8 | 7.9 |
| Range | | 4.1 - 8.00 | 4.9 - 10.0 | 7.0 - 8.7 | 6.4 - 9.7 |
| Av. food intake (gm./day) | | 20.6 | 21.4 | 33.3 | 36.0 |
| Range | | 18.9 - 22.0 | 16.7 - 25.4 | 30.1 - 48.4 | 31.7 - 44.6 |
| Av. wt. gain per gram food eaten (gm.) | | 0.29 | 0.31 | 0.24 | 0.16 |
| Range | | 0.10 - 0.42 | 0.24 - 0.45 | 0.18 - 0.29 | 0.16 - 0.28 |

Table 16. (Continued)

| Week of study | | V S"B | V S"A | V C | V CA |
|---------------|--|-------------|-------------|-------------|-------------|
| 7 | No. of animals | 5 | 6 | 6 | 6 |
| | Av. wt. at end of week (gm.) | 501 | 477 | 538 | 556 |
| | Range | 475 - 530 | 340 - 586 | 501 - 613 | 507 - 594 |
| | Av. wt. gain (gm./day) | 7.3 | 6.7 | 6.5 | 5.9 |
| | Range | 5.0 - 10.0 | 1.6 - 8.9 | 4.0 - 8.1 | 3.0 - 8.4 |
| | Av. food intake (gm./day) | 22.4 | 22.9 | 36.6 | 37.7 |
| | Range | 21.0 - 23.1 | 20.1 - 25.4 | 33.7 - 43.7 | 36.0 - 43.3 |
| | Av. wt. gain per gram food eaten (gm.) | 0.33 | 0.29 | 0.17 | 0.16 |
| | Range | 0.22 - 0.43 | 0.07 - 0.40 | 0.12 - 0.24 | 0.07 - 0.23 |
| 8 | No. of animals | 4 | 4 | 5 | |
| | Av. wt. at end of week (gm.) | 539 | 490 | 563 | |
| | Range | 502 - 558 | 342 - 610 | 555 - 610 | |
| | Av. wt. gain (gm./day) | 5.7 | 3.7 | 4.4 | |
| | Range | 3.0 - 8.0 | 0.3 - 7.9 | 0.4 - 8.6 | |
| | Av. food intake (gm./day) | 23.4 | 24.8 | 37.1 | |
| | Range | 21.3 - 24.4 | 24.6 - 25.1 | 31.0 - 42.6 | |
| | Av. wt. gain per gram food eaten (gm.) | 0.25 | 0.14 | 0.12 | |
| | Range | 0.12 - 0.34 | 0.00 - 0.28 | 0.00 - 0.22 | |

Figure 2. Average daily weight gains and food intakes of individual guinea pigs in the S"B and S"A groups and of these two groups of Experiment V



some rats were particularly resistant to biotin deficiency and even recovered spontaneously. Boas (1924b) retracted her original statement (Boas, 1924a) that dried egg white was harmful to rats when rats in the later experiment did not develop the symptoms observed earlier. Still later, Boas (1927) noted that rats were able to store the protective factor (biotin), and that the degree of this reserve in young rats depended not only on their own body weights, but also on the amount of the factor (biotin) in their previous diets and in the diets of the mothers.

Guinea pigs S"A10, 11, and 12 grew excellently and equaled the control group in every way (Tables 16 and 17 and Figure 2). Guinea pigs S"A9 and 13 grew somewhat more slowly during the experiment than S"A10, 11, and 12, while guinea pigs S"A8 and 14 both grew considerably more slowly and utilized their food less efficiently for growth than S"A10, 11, and 12. Guinea pig S"A8 lost 4 grams during the 5th week after 4 weeks of progressively smaller weight gains. Subsequently, it gained weight again, 4.9 grams per day in the 6th week, 1.6 grams per day in the 7th and 0.3 grams per day in the final week of the experiment. Its hepatic biotin concentration, 0.38 mcg. per gram, was the lowest biotin concentration observed up to this time. Guinea pig S"A14 died during the 6th week following 4 weeks of small weight gains and weight losses.

Table 17. Summary of weight gain, food intake, and food efficiency for guinea pigs during Experiment V

| Animal | Beginning weight (gm.) | Final weight (gm.) | No. of days on expt. | Final wt. increase (gm.) | Av. wt. gain (gm./day) | Av. food intake (gm./day) | Food efficiency (gm.) |
|---------|------------------------|--------------------|----------------------|--------------------------|------------------------|---------------------------|-----------------------|
| V S"B1 | 140 | 328 | 29 | 188 | 6.5 | 13.4 | 0.48 |
| V S"B3 | 182 | 575 | 60 | 393 | 6.6 | 18.0 | 0.37 |
| V S"B4 | 167 | 572 | 60 | 405 | 6.8 | 18.1 | 0.37 |
| V S"B5 | 193 | 515 | 52 | 322 | 6.2 | 18.8 | 0.33 |
| V S"B6 | 205 | 569 | 60 | 364 | 6.1 | 18.8 | 0.32 |
| V S"B7 | 207 | 537 | 60 | 330 | 5.5 | 17.8 | 0.31 |
| V S"A8 | 154 | 390 | 60 | 236 | 3.9 | 17.6 | 0.22 |
| V S"A9 | 159 | 471 | 60 | 312 | 5.2 | 14.9 | 0.35 |
| V S"A10 | 166 | 620 | 60 | 454 | 7.6 | 17.5 | 0.43 |
| V S"A11 | 191 | 642 | 60 | 451 | 7.5 | 19.9 | 0.38 |
| V S"A12 | 187 | 584 | 52 | 397 | 7.6 | 20.4 | 0.38 |
| V S"A13 | 192 | 450 | 52 | 258 | 5.0 | 16.9 | 0.29 |
| V S"A14 | 216 | 346 | 34 | 130 | 3.8 | 14.8 | 0.26 |
| V C15 | 157 | 615 | 61 | 458 | 7.5 | 29.1 | 0.26 |
| V C16 | 180 | 636 | 61 | 456 | 7.5 | 28.0 | 0.27 |
| V C17 | 160 | 500 | 50 | 340 | 6.8 | 25.7 | 0.27 |
| V C19 | 207 | 596 | 61 | 389 | 6.4 | 28.5 | 0.22 |
| V C20 | 219 | 636 | 61 | 417 | 6.8 | 31.9 | 0.21 |
| V C21 | 217 | 672 | 61 | 455 | 7.5 | 33.6 | 0.22 |
| V CA23 | 184 | 579 | 51 | 395 | 7.8 | 29.2 | 0.27 |
| V CA24 | 176 | 628 | 51 | 452 | 8.9 | 32.6 | 0.25 |
| V CA25 | 198 | 525 | 51 | 327 | 6.4 | 27.1 | 0.22 |
| V CA26 | 208 | 586 | 51 | 378 | 7.4 | 29.1 | 0.25 |
| V CA27 | 225 | 587 | 51 | 362 | 7.1 | 30.5 | 0.22 |
| V CA28 | 234 | 515 | 51 | 281 | 5.5 | 25.2 | 0.21 |

Total weight gains of guinea pigs of the S"B group were slightly lower than those of the C group (Table 17). There were several weeks when individual guinea pigs of the S"B group had low average daily gains (Figure 2). Guinea pig S"B4 had a cold during the 4th week which affected its rate of weight gain and food efficiency. Guinea pig S"B7 was nervous, frequently remaining huddled in a corner of its cage, and had periods of poor food and water consumption. Guinea pig S"B1 died during the 5th week of the experiment. While cause of death was not determined, weight gains and general appearance up to the time of death indicated that the diet was adequate.

The avidin preparation fed to guinea pigs receiving commercial rabbit pellets (3.0 mg. daily for the first 2 weeks, 6.0 mg. daily for the remainder of the experiment) did not affect their weight gains, food intakes, and food efficiencies (Tables 16 and 17).

Experiment VI

In Experiment VI, young guinea pigs, weighing 118 to 186 grams, received either synthetic diet plus biotin or synthetic diet plus avidin. Six milligrams of avidin preparation per day was fed for the first 10 days and 18 mg. per day for the remainder of the experiment.

Gains in weight by guinea pigs in the control group were

not as large as those of either the S"B or C groups in Experiment V, but were similar to those of the C group in Experiment IV. Comparing weight gains of the control and experimental groups in this experiment, differences between the two groups increased as the experiment progressed (Table 18). Initially guinea pigs in the avidin-fed group averaged 2 grams heavier than those receiving only the synthetic diet, but by the end of the first week the latter group averaged 21 grams heavier than the former. This difference reflected the average weight gains per day which were 4.7 and 1.4 grams for control (S"B) and experimental (S"A) groups, respectively. Food efficiencies during the first week were lower for the S"A group than for the S"B group. In addition to this lower food efficiency the daily food intake of the control and experimental groups differed by an average of 2.2 grams.

During the second week the two groups behaved more nearly alike and only one animal in the experimental group lost weight. The difference in average weight of the two groups increased slightly to 28 grams. Perhaps slow adjustment to diet plus weight losses occurring during shipping accounted for the variation within the two groups in the first week, rather than dietary modifications.

Weight losses (Figure 3) of control guinea pigs occurred infrequently and could be related to temporary illness. Guinea pig S"B6 and S"B10 had colds at the time they were

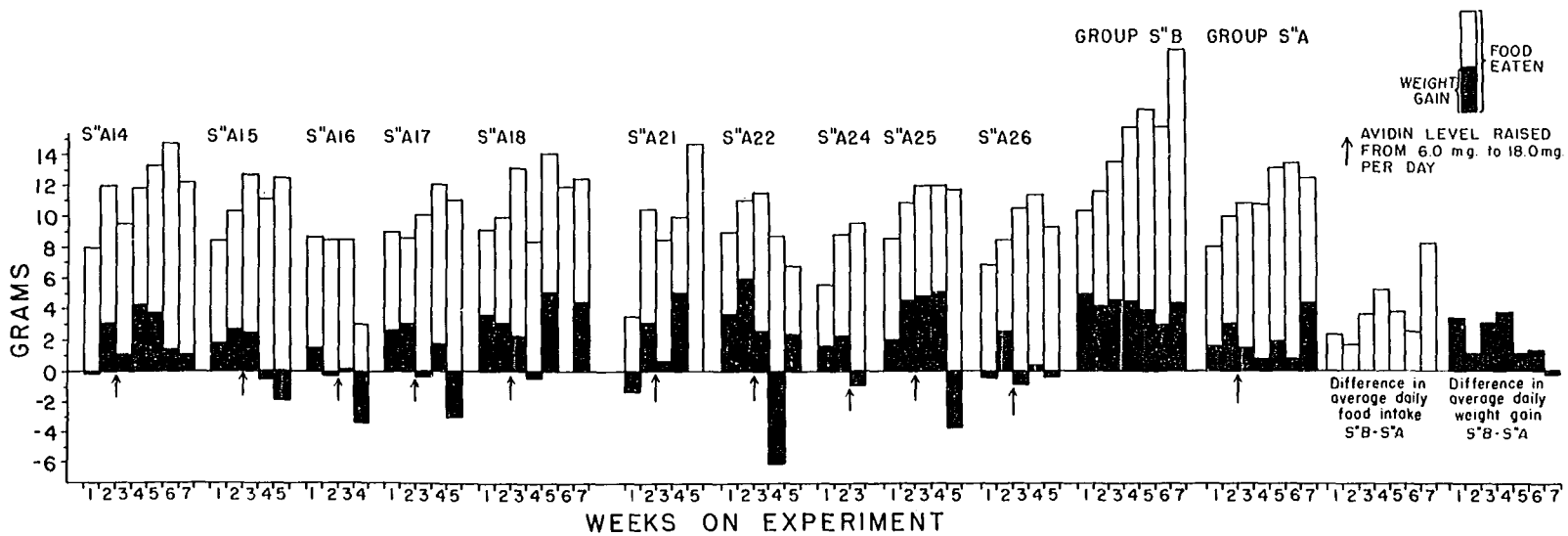
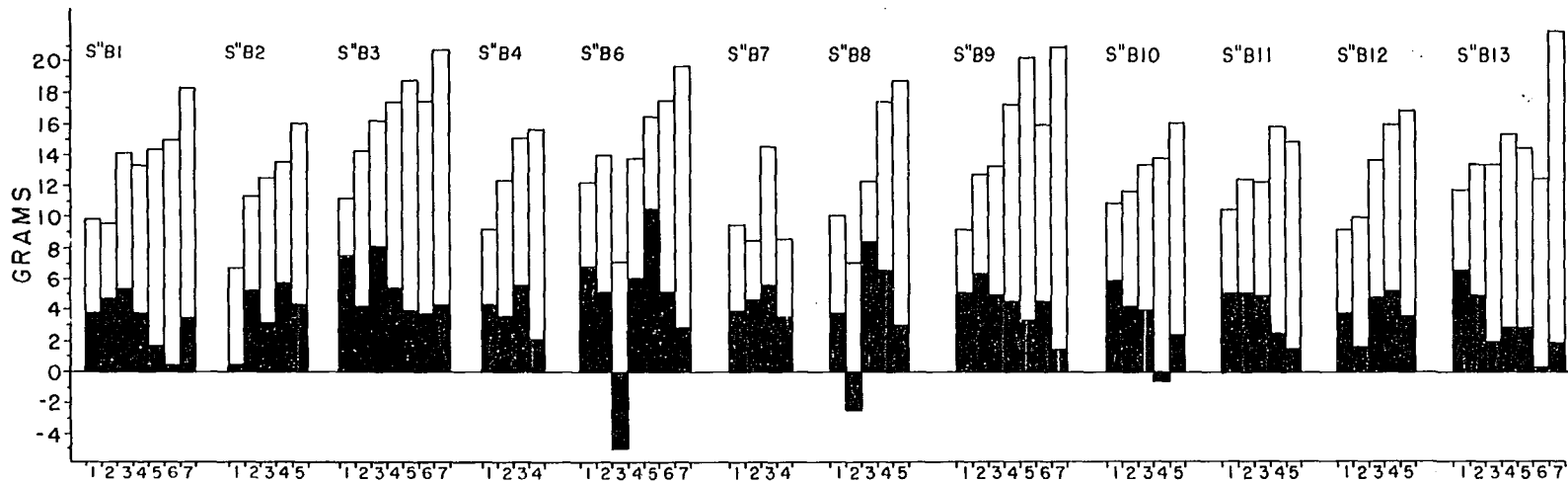
Table 18. Summary of weekly weight gain, food intake, and food efficiency of guinea pigs of Experiment VI

| Week of study | | VI S"B | VI S"A |
|--|--|----------------|--------------|
| 1 | No. of animals | 12 | 10 |
| | Av. initial wt. (gm.) | 146 | 148 |
| | Range | 118 - 163 | 122 - 186 |
| | Av. wt. at end of week (gm.) | 179 | 158 |
| | Range | 121 - 210 | 126 - 199 |
| | Av. wt. gain (gm./day) | 4.7 | 1.4 |
| | Range | 0.4 - 7.4 | -1.4 - 3.6 |
| | Av. food intake (gm./day) | 10.1 | 7.9 |
| | Range | 6.7 - 12.1 | 3.4 - 9.1 |
| | Av. wt. gain per gram food eaten (gm.) | 0.45 | 0.13 |
| Range | 0.07 - 0.66 | -0.41 - 0.40 | |
| 2 | No. of animals | 12 | 10 |
| | Av. wt. at end of week (gm.) | 206 | 178 |
| | Range | 156 - 246 | 147 - 230 |
| | Av. wt. gain (gm./day) | 3.9 | 2.9 |
| | Range | -2.6 - 6.3 | -0.1 - 5.9 |
| | Av. food intake (gm./day) | 11.5 | 9.9 |
| | Range | 7.0 - 14.3 | 8.4 - 12.0 |
| | Av. wt. gain per gram food eaten (gm.) | 0.32 | 0.30 |
| | Range | -0.37 - 0.49 | -0.02 - 0.53 |
| | 3 | No. of animals | 12 |
| Av. wt. at end of week (gm.) | | 236 | 191 |
| Range | | 177 - 286 | 150 - 263 |
| Av. wt. gain (gm./day) | | 4.3 | 1.3 |
| Range | | -5.4 - 8.3 | -1.0 - 2.4 |
| Av. food intake (gm./day) | | 13.3 | 10.7 |
| Range | | 6.9 - 14.5 | 8.6 - 13.1 |
| Av. wt. gain per gram food eaten (gm.) | | 0.28 | 0.11 |
| Range | | -0.75 - 0.67 | -0.04 - 0.40 |

Table 18. (Continued)

| Week of study | | VI S"B | VI S"A |
|---------------|---|--------------|--------------|
| 4 | No. of animals | 10 | 8 |
| | Av. wt. at end of week (gm.) | 256 | 191 |
| | Range | 227 - 323 | 150 - 263 |
| | Av. wt. gain (gm./day) | 4.2 | 0.5 |
| | Range | -0.4 - 6.6 | -6.1 - 5.0 |
| | Av. food intake (gm./day) | 15.7 | 10.6 |
| | Range | 13.3 - 17.4 | 8.3 - 11.9 |
| | Av. wt. gain per gram good eaten (gm.) | 0.27 | -0.02 |
| | Range | -0.01 - 0.42 | -0.42 - 0.39 |
| 5 | No. of animals | 8 | 3 |
| | Av. wt. at end of week (gm.) | 297 | 217 |
| | Range | 245 - 350 | 179 - 251 |
| | Av. wt. gain (gm./day) | 3.8 | 1.8 |
| | Range | 1.6 - 10.4 | -3.1 - 4.9 |
| | Av. food intake (gm./day) | 16.8 | 13.0 |
| | Range | 14.3 - 20.9 | 11.7 - 14.0 |
| 6 | Av. wt. gain per gram food eaten (gm.) | 0.24 | 0.09 |
| | Range | 0.10 - 0.64 | -0.29 - 0.35 |
| | No. of animals | 5 | 2 |
| | Av. wt. at end of week (gm.) | 331 | 240 |
| 7 | Range | 268 - 376 | 230 - 251 |
| | Av. wt. gain (gm./day) | 2.8 | 0.6 |
| | Range | 0.1 - 5.0 | 0.0 - 0.3 |
| | Av. food intake (gm./day) | 15.7 | 13.3 |
| | Range | 14.9 - 17.4 | 11.9 - 14.7 |
| | Av. wt. gain per gram food eaten (gm.) | 0.17 | 0.04 |
| | Range | 0.01 - 0.29 | 0.00 - 0.09 |
| 7 | No. of animals | 1 | 1 |
| | Av. wt. at end of week (gm.) | 405 | 281 |
| | Av. wt. gain (gm./day) | 4.1 | 4.3 |
| | Av. food intake (gm./day) | 20.7 | 12.4 |
| | Av. wt. gain per gram food eaten (gm.) | 0.20 | 0.34 |

Figure 3. Average daily weight gains and food intakes of individual guinea pigs and of groups of Experiment VI



losing weight and S"B8 appeared frightened at the time it was losing weight. Two other guinea pigs had periods of small weight gains during the time they were on experiment. S"B1 had a cold during the 5th and 6th weeks and S"B13 had a sore eye during the 6th week.

In the experimental group, weight losses were more frequent and weight gains were smaller than those of the control group. Guinea pig S"A14 gradually reduced its weight gain as the experiment progressed, but actual weight loss occurred only during the first week. All others had weeks with either weight losses or no net gains. S"A15 had increasing weight losses for the 2 weeks prior to autopsy. Cyclic patterns of weight gain and losses were noticed in guinea pigs S"A16, 17, 18, 22, and 26 during the experiment. (Figure 3) Guinea pigs S"A24 and 25 lost weight only in the last week prior to autopsy; S"A24 had gained weight moderately well and S"A25 very well before weight loss began.

Six out of 10 animals receiving avidin achieved their maximum weights between the 18th and 23rd day (Table 19). In addition, guinea pig S"A15, which achieved maximum weight on the 32nd day, was within 3 grams of its maximum weight about the 20th day. Its weight fluctuated but remained essentially constant from the 20th to the 32nd day.

Only guinea pigs S"A16 and 22 reduced their food intakes during the experiment; the remainder increased their average

Table 19. Summary of maximum weights and weight decreases of guinea pigs of Experiment VI

| Animal | Beginning weight (gm.) | Maximum weight (gm.) | Weight increase (gm.) | Days on expt. at time of highest wt. | Final weight (gm.) | Weight decrease (gm.) | Total no. days on expt. |
|----------|------------------------|----------------------|-----------------------|--------------------------------------|--------------------|-----------------------|-------------------------|
| VI S"B1 | 138 | 281 | 143 | 46 | 281 | - | 46 |
| VI S"B2 | 118 | 245 | 127 | 35 | 245 | - | 35 |
| VI S"B3 | 150 | 429 | 279 | 50 | 429 | - | 50 |
| VI S"B4 | 156 | 265 | 109 | 23 | 253 | 12 (4.7) ^a | 25 |
| VI S"B6 | 163 | 374 | 211 | 47 | 374 | - | 47 |
| VI S"B7 | 135 | 246 | 111 | 23 | 246 | - | 23 |
| VI S"B8 | 155 | 279 | 124 | 32 | 279 | - | 32 |
| VI S"B9 | 146 | 359 | 213 | 46 | 359 | - | 46 |
| VI S"B10 | 129 | 243 | 114 | 32 | 240 | 3 (1.2) | 33 |
| VI S"B11 | 141 | 282 | 141 | 35 | 282 | - | 35 |
| VI S"B12 | 162 | 295 | 133 | 35 | 295 | - | 35 |
| VI S"B13 | 163 | 316 | 153 | 47 | 316 | - | 47 |
| VI S"A14 | 136 | 240 | 104 | 45 | 234 | 6 (2.5) | 46 |
| VI S"A15 | 133 | 187 | 54 | 32 | 185 | 2 (1.1) | 33 |
| VI S"A16 | 173 | 196 | 23 | 19 | 176 | 20 (14.2) | 23 |
| VI S"A17 | 155 | 205 | 50 | 18 | 179 | 26 (12.7) | 35 |
| VI S"A18 | 160 | 283 | 123 | 48 | 281 | 2 (0.7) | 49 |
| VI S"A21 | 136 | 194 | 55 | 31 | 185 | 9 (4.6) | 32 |
| VI S"A22 | 122 | 205 | 83 | 21 | 169 | 36 (17.6) | 31 |
| VI S"A24 | 125 | 162 | 37 | 18 | 143 | 19 (11.7) | 20 |
| VI S"A25 | 186 | 266 | 80 | 23 | 209 | 57 (21.4) | 33 |
| VI S"A26 | 158 | 181 | 23 | 19 | 159 | 22 (12.2) | 32 |

^aPer cent weight loss

daily food consumptions in the later weeks of the experiment. When the food consumption of a biotin deficient guinea pig was compared with its food consumption earlier in the experiment, but at the same weight, it was always higher at the later time. Food intakes also nearly equaled those of control guinea pigs of similar weight. For example, during the 5th week, experimental animals ranged in weight from 179 to 251 grams and in food intake from 11.7 to 14.0 grams (Table 18). Control animals during the 3rd week ranged in weight from 177 to 286 grams and in food intake from 6.9 to 14.5 grams. The increasing difference between food intakes of control and experimental groups resulted from a slower rate of increase rather than a decrease in food consumption as the deficiency developed.

In evaluating autopsy findings and composition of different tissues, an effort has been made to distinguish direct effects of biotin deficiency from indirect effects of reduced food intake and weight loss. The extent of inanition observed in biotin-deficient guinea pigs of Experiment VI was difficult to assess since they gained and lost weight irregularly, and biotin-deficient guinea pigs perhaps were not utilizing ingested food efficiently, hence starving their tissues. In Experiment VI, the weight loss of guinea pigs in the S^A group varied from 0.7 to 21.4 per cent of maximum body weight (Table 19).

Data for individual guinea pigs are presented in Table 20. Both weight gain and food efficiency were reduced in the experimental group when compared with the control group. The latter averaged 4.0 grams weight gain per day over the entire experimental period while the former averaged 1.2 grams. The extreme values for the two groups were 3.1 and 5.6 grams per day in controls and 0.0 and 2.5 grams per day in the experimental group. Food efficiencies ranged from 0.22 to 0.46 for the control group and 0.00 to 0.22 for the experimental group.

Retarded growth or weight loss invariably accompanied by reduced food intake has been reported for biotin-deficient rats (Boas, 1927; Parsons and Lease, 1934; and Katsh et al., 1955). Weight loss has been attributed partly to inanition and partly to biotin deficiency since rats receiving egg white in their diet had lesser weight increases than rats pair-fed the same amount of egg white diet plus biotin (Katsh et al., 1955). Coots et al. (1959) noted failure to grow and weight loss in young adult guinea pigs and weight loss in adult guinea pigs, but no information was given on food intakes.

In summary, in the sixth experiment the feeding of avidin as a crude extract produced a marked effect on growth of guinea pigs. This reduced rate of growth compared to controls fed the same diet without avidin, but with biotin, was the first effect observed as a result of the dietary modification.

Table 20. Summary of weight gain, food intake, and food efficiency for guinea pigs during Experiment VI

| Animal | Beginning weight (gm.) | Final weight (gm.) | No. of days on expt. | Final wt. increase (gm.) | Av. wt. gain (gm./day) | Av. food intake (gm./day) | Food efficiency (gm.) |
|----------|------------------------|--------------------|----------------------|--------------------------|------------------------|---------------------------|-----------------------|
| VI S"B1 | 138 | 281 | 46 | 143 | 3.1 | 13.1 | 0.24 |
| VI S"B2 | 118 | 245 | 35 | 127 | 3.6 | 12.0 | 0.30 |
| VI S"B3 | 150 | 429 | 50 | 279 | 5.6 | 16.6 | 0.34 |
| VI S"B4 | 156 | 253 | 25 | 97 | 3.9 | 13.1 | 0.30 |
| VI S"B6 | 163 | 374 | 47 | 211 | 4.5 | 14.1 | 0.32 |
| VI S"B7 | 135 | 246 | 23 | 111 | 4.8 | 10.4 | 0.46 |
| VI S"B8 | 155 | 279 | 32 | 124 | 3.9 | 12.6 | 0.31 |
| VI S"B9 | 146 | 359 | 46 | 213 | 4.6 | 15.5 | 0.30 |
| VI S"B10 | 129 | 240 | 33 | 111 | 3.4 | 13.1 | 0.26 |
| VI S"B11 | 141 | 382 | 35 | 141 | 4.0 | 13.3 | 0.30 |
| VI S"B12 | 162 | 295 | 35 | 133 | 3.8 | 13.2 | 0.29 |
| VI S"B13 | 163 | 316 | 47 | 153 | 3.3 | 14.6 | 0.22 |
| VI S"A14 | 136 | 242 | 46 | 98 | 2.1 | 11.6 | 0.18 |
| VI S"A15 | 133 | 185 | 33 | 52 | 1.6 | 10.9 | 0.14 |
| VI S"A16 | 173 | 176 | 23 | 3 | .1 | 8.1 | 0.02 |
| VI S"A17 | 155 | 179 | 35 | 24 | .7 | 10.2 | 0.07 |
| VI S"A18 | 160 | 281 | 59 | 121 | 2.5 | 11.2 | 0.22 |
| VI S"A21 | 136 | 185 | 32 | 49 | 1.5 | 8.4 | 0.18 |
| VI S"A22 | 122 | 169 | 31 | 47 | 1.5 | 9.7 | 0.16 |
| VI S"A24 | 125 | 143 | 20 | 18 | .9 | 8.7 | 0.10 |
| VI S"A25 | 186 | 209 | 33 | 23 | .7 | 10.9 | 0.06 |
| VI S"A26 | 158 | 159 | 32 | 1 | .0 | 9.2 | 0.00 |

Growth ceased in most instances, maximal weight being reached for the majority of the experimental guinea pigs between the 18th and 23rd day. Weight loss of varying severity followed as the experiment was continued.

Food efficiencies and weight gains
for Experiments I through VI

An overall evaluation of the effect of biotin deficiency on food efficiency was more meaningful when groups from the six different experiments were compared by their approximate ages as well as by length of time on experiment since animals varied in their initial weight and age (Table 21 and Figure 4). A gradual decrease in food efficiency as animals became older and grew more slowly was evident in all groups.

During the first week of each experiment food efficiency figures tended to be very irregular and during the second week were frequently higher than during the first. Some animals accepted the diet slowly resulting in weight losses, while others gained weight rapidly for the first 2 or 3 days probably because they were dehydrated during shipping. Only in group II S'S did the guinea pigs as a group give any indication of difficulty in adjusting to the diet.

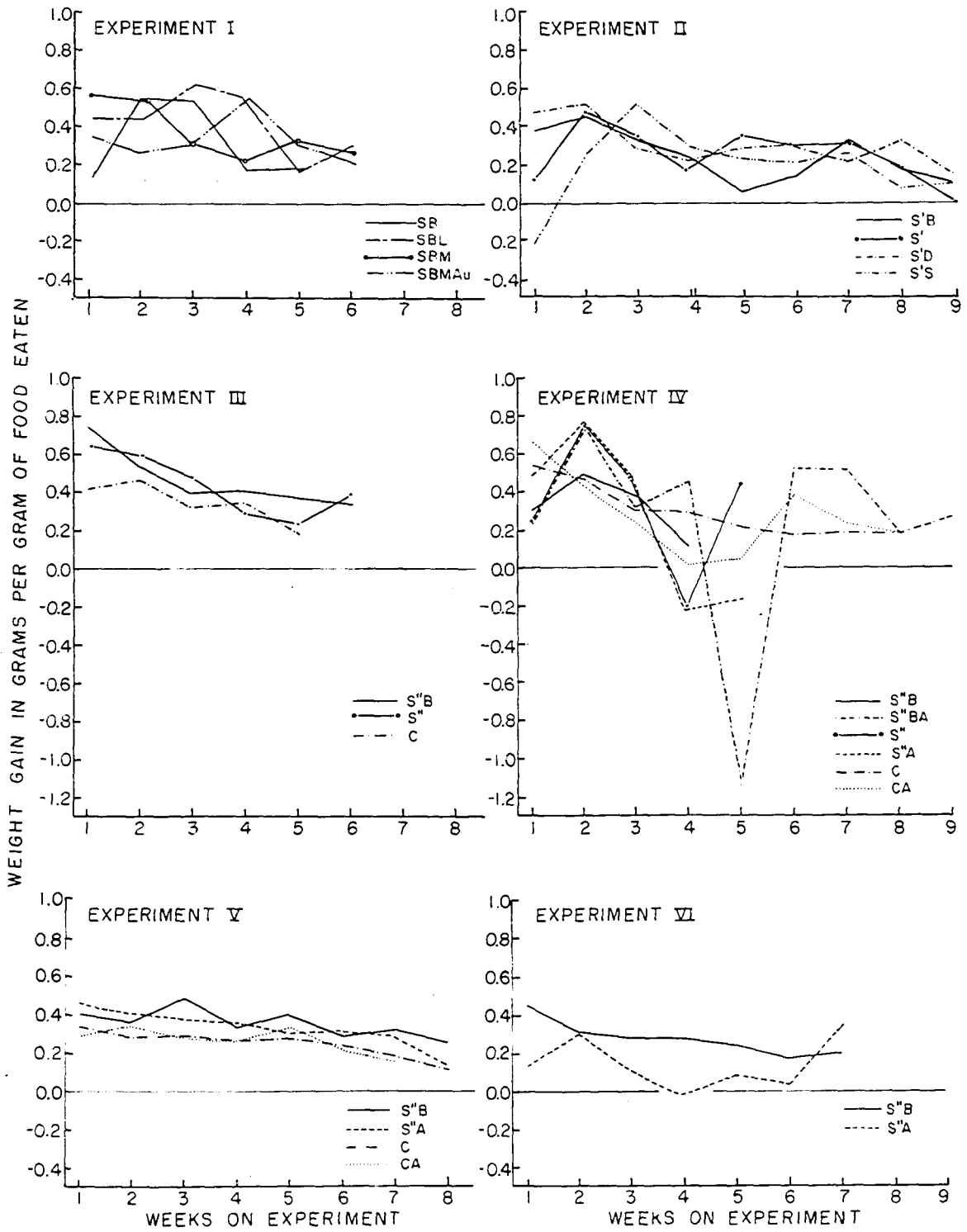
In Experiment III food efficiencies for groups receiving the synthetic diet either with or without biotin were very similar. Food efficiencies for the III C group were somewhat lower than the group receiving the synthetic diet, but this

Table 21. Average food efficiencies (grams gained per gram of food eaten) of groups of guinea pigs of Experiments I through VI

| Group | Age in weeks | | | | | | | | | | | |
|---------|--------------|-------------------|-------------------|-------|-------|------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| I SB | | | 0.13 | 0.54 | 0.53 | 0.17 | 0.18 | 0.21 | | | | |
| I SBL | | | 0.44 | 0.43 | 0.62 | 0.56 | 0.16 | 0.30 | | | | |
| I SBM | | | 0.56 | 0.53 | 0.31 | 0.21 | 0.32 | 0.26 | | | | |
| I SBMAu | | | 0.34 | 0.25 | 0.31 | 0.54 | 0.30 | 0.20 | | | | |
| II S'B | | | 0.37 | 0.45 | 0.32 | 0.23 | 0.05 | 0.14 | 0.33 | 0.17 | 0.11 | 0.11 |
| II S' | | | 0.12 | 0.48 | 0.35 | 0.17 | 0.35 | 0.29 | 0.31 | 0.18 | 0.02 | 0.27 |
| II S'D | | | 0.48 | 0.52 | 0.28 | 0.22 | 0.28 | 0.30 | 0.21 | 0.33 | 0.16 | |
| II S'S | | | -0.22 | 0.25 | 0.52 | 0.29 | 0.23 | 0.21 | 0.26 | 0.08 | 0.09 | |
| III S"B | | 0.74 | 0.53 | 0.39 | 0.40 | 0.37 | 0.34 | | | | | |
| III S" | | 0.64 | 0.60 | 0.48 | 0.29 | 0.23 | 0.39 | | | | | |
| III C | | 0.42 | 0.46 | 0.32 | 0.34 | 0.19 | | | | | | |
| IV S"B | 0.30 | 0.50 | 0.37 | 0.11 | | | | | | | | |
| IV S"BA | 0.23 | 0.73 | 0.32 ^a | 0.46 | -1.14 | 0.53 | 0.52 | 0.19 | 0.27 | | | |
| IV S" | 0.23 | 0.73 | 0.48 | -0.22 | 0.45 | | | | | | | |
| IV S"A | 0.49 | 0.76 | 0.49 ^a | -0.21 | -0.16 | | | | | | | |
| IV C | 0.54 | 0.47 | 0.31 | 0.29 | 0.22 | 0.18 | 0.19 | 0.20 | | | | |
| IV CA | 0.66 | 0.44 | 0.24 ^a | 0.02 | 0.05 | 0.38 | 0.24 | 0.20 | | | | |
| V S"B | | | 0.40 ^a | 0.36 | 0.48 | 0.34 | 0.40 | 0.29 | 0.33 | 0.25 | | |
| V S"A | | | 0.46 ^a | 0.41 | 0.37 | 0.36 | 0.30 | 0.31 | 0.29 | 0.14 | | |
| V C | | | 0.34 | 0.28 | 0.29 | 0.26 | 0.27 | 0.24 | 0.18 | 0.12 | | |
| V CA | | | 0.29 | 0.34 | 0.28 | 0.26 | 0.32 | 0.22 | 0.16 | | | |
| VI S"B | | 0.45 | 0.31 | 0.28 | 0.27 | 0.24 | 0.17 | 0.20 | | | | |
| VI S"A | | 0.13 ^a | 0.30 | 0.11 | -0.02 | 0.09 | 0.04 | 0.34 | | | | |

^aAvidin feeding begun

Figure 4. Average weekly food efficiencies (weight gain in grams per gram of food eaten) of groups of guinea pigs in Experiments I through VI



can be explained by the lower caloric density of the commercial rabbit chow than the synthetic diet.

In Experiment IV there were wide variations in food efficiencies of groups. Since few or single animals made up the groups, weight loss and low food intake of a single animal affected the average of the group and explained in part the great irregularity. This combination of weight loss and poor food intake resulted in a food efficiency of -1.14 during the 5th week for the only guinea pig in group IV S"BA. Weight losses during the 4th and 5th weeks for the only guinea pig in the IV S"A group also resulted in negative food efficiencies. The third guinea pig receiving avidin, IV CA, had low food efficiencies for the 4th and 5th weeks. Guinea pig IV S"A did have a low concentration of biotin in its liver, while IV S"BA and IV CA did not. However, it was possible that a biotin deficiency was beginning in the latter two guinea pigs at the time that low food efficiencies were obtained. These two guinea pigs were autopsied several weeks later and concentrations of biotin in the liver could have increased during the intervening weeks when their food consumption had increased.

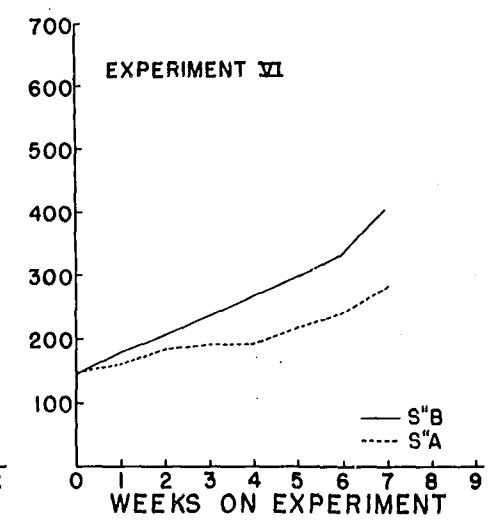
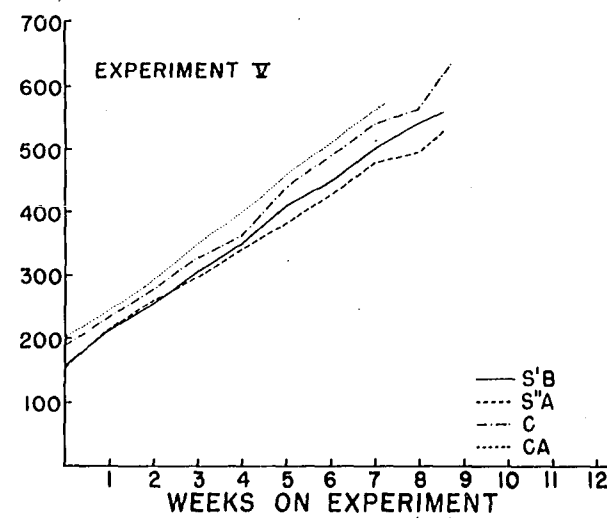
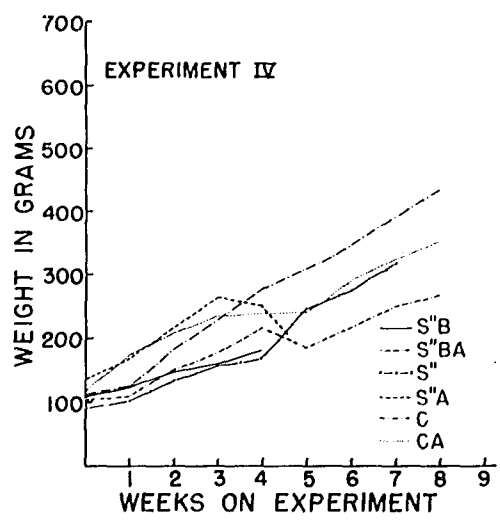
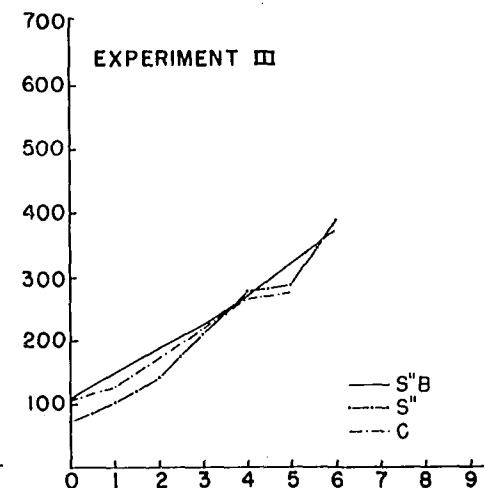
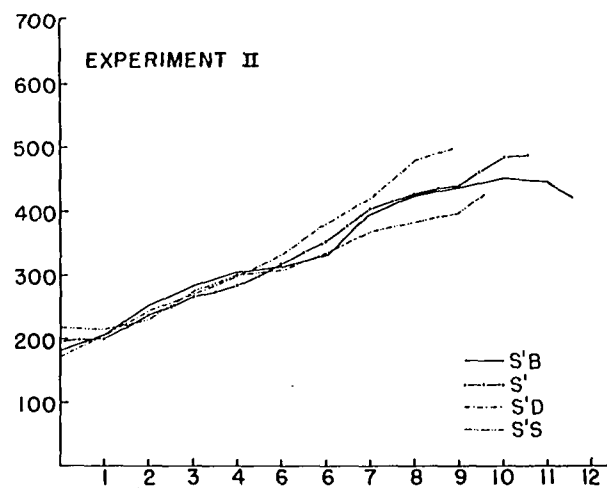
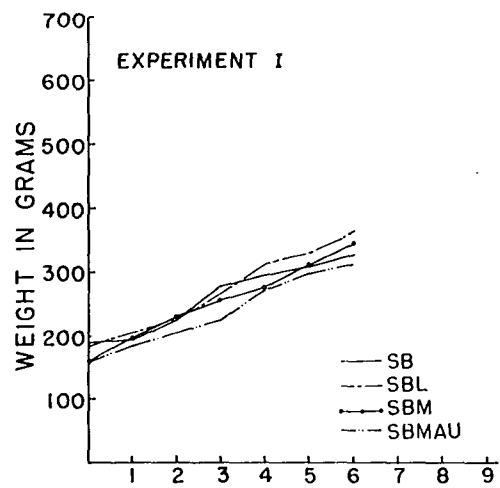
The S"B and S"A groups in Experiment V had very similar food efficiencies as did groups C and CA. Thus avidin did not affect the average food efficiency of the groups in this experiment. Although low food efficiencies were found in two

guinea pigs in the S"A group, there were also high food efficiencies in others.

In Experiment VI a difference in food efficiency between the control and avidin-fed groups began during the third week of the experiment. Many avidin-fed guinea pigs lost weight and were autopsied during the 4th and 5th weeks and since weekly averages were calculated only for animals completing each week, the last days on experiment usually were not included. Data for the 7th week of the experiment were obtained from one guinea pig in each group. Cyclic weight losses and gains produced alternately high and low food efficiency values for the guinea pig in the S"A group and it happened to have a higher food efficiency in the 7th week than the remaining guinea pig in the S"B group.

A weekly summary of the average weights for all groups in all experiments is presented in Figure 5. Reduction in rate of weight gain during biotin deficiency is clearly shown for the two guinea pigs IV S"A and IV S"BA and for the VI S"A group. Weights of groups receiving the synthetic diet, synthetic diet plus desthiobiotin, or synthetic diet plus sulfasuxidine were unaffected by these dietary modifications. Although individual guinea pigs in the synthetic diet groups of Experiments III and IV and the S"A group of Experiment V had reduced weight gains, this behavior did not affect the average of the group.

Figure 5. Average weekly weights of groups of guinea pigs in Experiments I through VI



Findings of Autopsies and Analyses

Many guinea pigs being fed synthetic diet developed enlarged abdomens during the experiments. At autopsy an accumulation of food in the gastrointestinal tract, particularly in the cecum of these animals was observed. Enlarged abdomens also have been observed by Reid and Briggs (1953). Because accumulation of food within the gastrointestinal tract was variable, comparisons of organ weight-body weight ratios based on the final live weight of the animals did not reflect the relationship of organs to active tissues. Hence, the gastrointestinal tract, beginning with the esophagus at a point immediately below the diaphragm, was removed and weighed and its weight subtracted from the weight of the intact animal. The value for body weight minus gastrointestinal tract, therefore, included such tissues as lung, liver, kidney, heart, blood, and reproductive organs.

The per cent of body weight due to the gastrointestinal tract was calculated for Experiments II through VI; it varied within groups and between similar diet groups in different experiments, and accounted for from 1/8 to 1/5 of the intact body weight. See Appendix Table 36.

Hematological studies

Concentrations of hemoglobin and volume of red blood cells did not decrease consistently in biotin-deficient guinea

pigs (Tables 22 and 23). This might be due in part to the decreased demand for new circulating red blood cells due to diminished volume of blood required in biotin-deficient guinea pigs which were losing weight, while control guinea pigs had greater demands because of their increasing size.

Guinea pigs in Experiment V which exhibited lower biotin concentrations in their livers or slower growth rates, did not differ from controls in hemoglobin concentrations and packed red blood cells volume. Guinea pig S"A8, which had the lowest concentration of biotin in its liver and the smallest weight increase for this experiment, had normal values for hemoglobin and packed red blood cell volume. Guinea pigs S"A9 and 13, which had slightly reduced biotin concentrations in the liver, had relatively high values for hemoglobin and red blood cell volume.

Hemoglobin concentrations and red blood cell volumes of all guinea pigs in Experiment V were slightly higher than those found in control guinea pigs in Experiments I through IV. In the first four experiments, hemoglobin concentration ranged from about 11.00 to 13.25 grams per 100 ml. of blood and the packed red blood cell volumes ranged from 32.0 to 40.0 per cent. These values were lower than those reported for guinea pigs by other workers. Cannon (1954) had found an average hemoglobin concentration of 14.00 grams per 100 ml. of blood for guinea pigs fed a diet of crude natural mate-

Table 22. Packed red blood cell volume, and concentration of hemoglobin, urea nitrogen, and nonprotein nitrogen in the blood of guinea pigs of Experiment V

| Animal | Packed red blood cell volume (per cent) | Hemo- globin (gm./ 100 ml.) | Urea nitro- gen (mg./ 100 ml.) | Non- protein nitrogen (mg./ 100 ml.) | $\frac{\text{Urea N}}{\text{NPN}} \times 100$ |
|---------|--|--------------------------------------|--|--|---|
| V S"B1 | - | - | - | - | - |
| V S"B3 | 37.6 | 12.62 | 26.5 | 40.2 | 64.9 |
| V S"B4 | 43.8 | 12.89 | 26.0 | 42.5 | 61.2 |
| V S"B5 | 42.3 | 11.97 | 25.2 | 40.2 | 62.8 |
| V S"B6 | 37.7 | 12.60 | 26.8 | 39.2 | 68.2 |
| V S"B7 | 38.4 | 12.23 | 23.8 | 34.2 | 69.6 |
| Average | 40.0 | 12.46 | 25.7 | 39.4 | 65.4 |
| V S"A8 | 35.4 | 11.52 | 23.8 | 30.8 | 77.2 |
| V S"A9 | 40.4 | 14.28 | 27.5 | 35.6 | 77.2 |
| V S"A10 | 35.2 | 11.15 | 21.1 | 34.3 | 61.5 |
| V S"A11 | 40.4 | 12.57 | 26.8 | 34.8 | 77.0 |
| V S"A12 | 39.7 | 12.51 | 32.2 | 38.6 | 83.4 |
| V S"A13 | 38.8 | 13.05 | 27.8 | 33.0 | 84.1 |
| V S"A14 | - | - | - | - | - |
| Average | 38.3 | 12.51 | 26.6 | 34.6 | 76.8 |
| V C15 | 40.6 | 12.49 | 21.0 | 38.4 | 54.8 |
| V C16 | 32.1 | 11.53 | 19.6 | 35.6 | 55.0 |
| V C17 | 36.3 | 12.14 | 17.8 | 36.2 | 49.3 |
| V C19 | 43.6 | 13.94 | 20.2 | 38.4 | 52.7 |
| V C20 | 40.8 | 13.86 | 23.2 | 41.5 | 55.9 |
| V C21 | 41.2 | 13.28 | 22.8 | 38.0 | 60.0 |
| Average | 39.1 | 12.87 | 20.8 | 38.0 | 54.6 |
| V CA23 | 42.6 | 12.58 | 10.2 | 36.8 | 27.8 |
| V CA24 | 39.5 | 13.10 | 10.1 | 30.2 | 23.6 |
| V CA25 | 40.2 | 12.96 | 9.9 | 37.0 | 26.8 |
| V CA26 | 41.8 | 12.99 | 18.9 | 36.0 | 52.4 |
| V CA27 | 36.5 | 11.76 | 17.0 | 33.2 | 51.1 |
| V CA28 | 34.5 | 11.69 | 10.4 | 29.2 | 35.5 |
| Average | 39.2 | 12.51 | 12.8 | 33.8 | 38.9 |

Table 23. Packed red blood cell volume, and concentration of hemoglobin, urea nitrogen, and nonprotein nitrogen in the blood of guinea pigs of Experiment VI

| Animal | Packed red blood cell volume (per cent) | Hemo-globin (gm./100 ml.) | Urea nitro-gen (mg./100 ml.) | Non-protein nitrogen (mg./100 ml.) | $\frac{\text{Urea N}}{\text{NPN}} \times 100$ |
|----------|---|---------------------------|------------------------------|------------------------------------|---|
| VI S"B1 | 35.4 | 11.39 | 15.6 | 39.1 | 39.8 |
| VI S"B2 | 34.6 | 11.42 | 30.8 | 49.5 | 62.2 |
| VI S"B3 | 34.0 | 11.46 | 14.1 | 36.4 | 38.7 |
| VI S"B4 | 36.4 | 11.59 | 19.6 | 42.8 | 45.7 |
| VI S"B6 | 35.9 | 21.00 | 19.6 | 33.6 | 58.3 |
| VI S"B7 | 36.3 | 11.44 | 18.9 | 33.2 | 56.8 |
| VI S"B8 | 32.1 | 10.82 | 20.7 | 37.6 | 55.0 |
| VI S"B9 | 35.2 | 11.66 | 19.8 | 33.3 | 59.6 |
| VI S"B10 | 35.5 | 10.86 | 20.5 | 38.6 | 53.1 |
| VI S"B11 | 35.3 | 11.43 | 16.8 | 37.7 | 44.6 |
| VI S"B12 | 33.3 | 10.88 | 17.8 | 35.4 | 50.2 |
| VI S"B13 | 35.4 | 11.76 | 23.1 | 34.2 | 67.4 |
| Average | 35.0 ±1.26 ^a | 11.39 ± 0.36 | 19.8 ±4.23 | 37.6 ±4.71 | 52.6 ±8.99 |
| VI S"A14 | 34.6 | 11.37 | 20.6 | 32.4 | 63.5 |
| VI S"A15 | 42.1 | 12.41 | 23.4 | 35.1 | 66.5 |
| VI S"A16 | 35.1 | 11.42 | 16.8 | 42.9 | 39.2 |
| VI S"A17 | 29.7 | 9.97 | 17.0 | 38.0 | 44.9 |
| VI S"A18 | 35.4 | 11.97 | 19.8 | 49.2 | 40.3 |
| VI S"A21 | - | - | - | - | - |
| VI S"A22 | 49.9 | 14.46 | 19.8 | 46.6 | 42.6 |
| VI S"A24 | 34.3 | 11.44 | 23.5 | 61.2 | 38.4 |
| VI S"A25 | 28.4 | 8.78 | 20.0 | 39.0 | 51.2 |
| VI S"A26 | 35.6 | 11.02 | 27.4 | 43.8 | 62.4 |
| Average | 36.0 ±6.46 | 11.42 ±1.57 | 20.9 ±3.36 | 43.1 ±8.65 | 49.9 ±11.38 |

^aStandard deviation

rials. Woodruff (1953) obtained a similar average, 13.95 grams per 100 ml., for guinea pigs receiving a commercial diet, and Reid and Briggs (1953) observed an average hemoglobin concentration of 14.7 grams per 100 ml. of blood for 62-day-old guinea pigs fed a synthetic diet. Neither Cannon (1945) nor Reid and Briggs (1953) reported their methods for hemoglobin determinations. Woodruff (1953) used the same oxyhemoglobin method which was used in the present study.

In the sixth experiment, hemoglobin concentrations and red blood cell volumes observed in both control and experimental groups were not only lower than in Experiment V, but also lower than in the Experiments I through IV. Although averages for the two groups in Experiment VI were very similar, a wider range of values was found in biotin-deficient animals than in controls. Guinea pigs S[#]A17 and 25 both had very low hemoglobin concentrations, 9.97 grams per cent and 8.78 grams per cent, respectively, and red blood cell volumes, 29.7 per cent and 28.4 per cent, respectively, suggestive of anemia. The high concentrations of hemoglobin, 14.46 grams per cent, and red blood cell volume, 49.9 per cent, in S[#]A22 perhaps may be attributed to hemoconcentration because it drank little water prior to its collapse. Hemoconcentration has been observed in animals subjected to severe stress (Selye, 1937).

Anemia has been reported in biotin-deficient humans by

Sydenstricker et al. (1942a) who observed a definite diminution in hemoglobin content and volume of packed red cells in four human subjects. Only a few workers have measured hemoglobin concentrations in biotin-deficient rats. Parsons and Lease (1934) noted that rats suffering from dermatitis due to egg white ingestion showed no decrease either in total solids or in the hemoglobin content of the blood while Salmon and Goodman (1934) stated that in advanced cases some anemia was found.

Nonprotein nitrogen concentrations in whole blood of guinea pigs of Experiments V and VI varied considerably, but with the exception of VI S"A24 were within 30 to 51 mg. per cent nitrogen, the range given in The Handbook of Biological Data (Spector, 1956) as normal for plasma of guinea pigs. Since present determinations were made on protein-free filtrates of whole blood, somewhat higher values than those made on plasma might be expected; higher concentrations of nonprotein nitrogen are found in corpuscles than in plasma.

Although the feeding of avidin in Experiment V appeared to reduce slightly nonprotein nitrogen in the blood, findings in Experiment VI did not confirm this observation. The blood of guinea pig VI S"A24 contained 61.2 mg. of nonprotein nitrogen per 100 ml. while the blood of others contained the same amount of nonprotein nitrogen as their controls. Guinea pig VI S"A24 was also one of three guinea pigs in the avidin-fed

group that appeared to have areas of fatty infiltration penetrating the kidney cortex. Since the other two guinea pigs, S"A16 and 18, had normal nonprotein nitrogen values this kidney change did not necessarily cause the one instance of increased nonprotein nitrogen concentration.

Urea concentrations in whole blood of guinea pigs of Experiments V and VI, with the exception of guinea pig V S"A13, were within 8 to 28 mg. per cent nitrogen, the normal range in serum of guinea pigs cited by Spector (1956) in The Handbook of Biological Data. Since urea is freely diffusible through cell membranes, concentrations within the corpuscular and serum fractions of blood are similar. The rabbit pellets produced significantly lower urea nitrogen than synthetic diet in Experiment V, but the S"B and S"A groups of Experiment VI both had average concentrations similar to that of the pellet control group of Experiment V. Although in Experiment V, the avidin seemed to lower the urea nitrogen in the group receiving rabbit pellets, it did not in the groups receiving synthetic diet in either Experiments V or VI. Thus, even when severe deficiencies developed, urea nitrogen concentrations indicated neither alterations in protein metabolism nor impairment of kidney function.

The urea content of blood varies in proportion to the rate at which it is formed as a result of protein catabolism, and inversely, within certain limitations, to urine volume.

Since similar factors affect urea and nonprotein nitrogen concentrations, under most conditions the two are parallel. The per cent of nonprotein nitrogen due to urea nitrogen varied considerably between groups in Experiment V and within groups in Experiment VI, but it was not related to biotin deficiency.

On the basis of our present knowledge of the biochemical role of biotin in mammalian protein metabolism, only indirect changes would have been expected in biotin deficiency. An increase in nonprotein nitrogen could result if degenerative changes were present in the kidney. Urea and nonprotein nitrogen concentrations might also increase during severe weight loss, but in Experiment VI weight losses of guinea pigs of the S^A group were not associated with increases in nonprotein nitrogen concentrations in the blood. Increased activity of the adrenal glands in response to the stress of biotin deficiency might result in increased glucocorticoid secretion which would cause more rapid than normal deamination of amino acids in the body and, therefore, increased concentrations of blood urea. Although adrenal changes were observed in biotin deficient guinea pigs, these changes did not influence blood nonprotein nitrogen concentrations.

Liver size and composition

Amounts of fat, nitrogen, and moisture in the liver have been measured frequently as a means of observing gross alterations in metabolism due to dietary inadequacies.

Size Livers of biotin-deficient guinea pigs were smaller than those of their controls. Not only was the absolute size smaller, which was expected because of the smaller body size of the deficient guinea pig, but the liver weight to body weight ratios were smaller in the deficient guinea pigs than in the controls (Table 24). This ratio for the VI S"A group averaged 0.0429 for intact body weight, or 0.0579 for body weight minus gastrointestinal tract, while their controls averaged 0.0505, or 0.0680.

Reduction in liver size may have been due to reduced food intake rather than to biotin deficiency. Keys et al. (1950) and Jackson (1925) have reported that inanition markedly reduced the weight of the liver, but variability in individual data made interpretation difficult. Liver weight-body weight ratios differed due to age and other physiological conditions as well as to type and degree of inanition (Jackson, 1925). Loss of hepatic tissue usually was relatively greater than that of the body as a whole, especially in mature animals, but in the young, hepatic tissue was often lost to a lesser degree, the persistent growth impulse of the liver

Table 24. Liver weights and liver weight-body weight ratios for guinea pigs of Experiment VI

| Animal | Final weight (gm.) | Final weight minus gastrointestinal tract (gm.) | Weight of liver (gm.) | Ratio of liver weight to body weight | Ratio of liver weight to body weight minus gastrointestinal tract |
|----------|--------------------|---|-----------------------|--------------------------------------|---|
| VI S"B1 | 281 | 200 | 16.15 | 0.0575 | 0.0808 |
| VI S"B2 | 245 | 182 | 14.1 | 0.0576 | 0.0775 |
| VI S"B3 | 429 | 358 | 26.25 | 0.0612 | 0.0733 |
| VI S"B4 | 253 | 189 | 11.50 | 0.0455 | 0.0608 |
| VI S"B6 | 374 | 252 | 13.8 | 0.0369 | 0.0548 |
| VI S"B7 | 246 | 155 | 10.0 | 0.0406 | 0.0645 |
| VI S"B8 | 279 | 193 | 13.2 | 0.0473 | 0.0684 |
| VI S"B9 | 359 | 243 | 18.25 | 0.0508 | 0.0645 |
| VI S"B10 | 240 | 179 | 11.6 | 0.0483 | 0.0648 |
| VI S"B11 | 282 | 213 | 15.85 | 0.0562 | 0.0744 |
| VI S"B12 | 295 | 213 | 15.45 | 0.0524 | 0.0725 |
| VI S"B13 | 316 | 238 | 16.5 | 0.0522 | 0.0693 |
| | | | Average | 0.0505±0.0072 ^a | 0.0680±0.0072 |
| VI S"A14 | 234 | 188 | 10.0 | 0.0427 | 0.0532 |
| VI S"A15 | 185 | 143 | 10.7 | 0.0578 | 0.0748 |
| VI S"A16 | 176 | 111 | 6.5 | 0.0369 | 0.0464 |
| VI S"A17 | 179 | 110 | 7.9 | 0.0441 | 0.0725 |
| VI S"A18 | 281 | 224 | 11.3 | 0.0420 | 0.0507 |
| VI S"A21 | 185 | - | - | - | - |
| VI S"A22 | 169 | 118 | 5.35 | 0.0317 | 0.0453 |
| VI S"A24 | 143 | 111 | 7.2 | 0.0503 | 0.0649 |
| VI S"A25 | 209 | 152 | 8.3 | 0.0397 | 0.0546 |
| VI S"A26 | 159 | 110 | 6.45 | 0.0406 | 0.0586 |
| | | | Average | 0.0429±0.0076 | 0.0579±0.0107 |

^aStandard deviation

perhaps preventing weight loss from occurring.

In his book, Jackson (1925) reviewed several studies on effects of fasting on the livers of guinea pigs. When livers lost 52 per cent in weight along with an average loss of about 40 per cent in body weight, they appeared anemic and reddish yellow in color. The rate of liver weight losses was greatest during the earliest stages of inanition but varied at different stages of inanition. Loss of hepatic tissue was relatively less than that of the entire body in the later stages of inanition. Sequential changes in liver weight and body weight are summarized in Table 25.

Pecora and Highman (1953) observed that the liver weight-body weight ratios of thiamine-deficient rats were smaller than the ratios of their ad libitum-fed controls, but similar to those of pair-fed controls. Hence, as in thiamine deficiency, the low liver-body weight ratios found in biotin-deficient guinea pigs may have been the result of reduced food intake.

Table 25. Relationship of liver weight losses to body weight losses

| Per cent body weight loss | $\frac{\text{Liver}}{\text{Body}} \times 100$ |
|---------------------------|---|
| 0.00 | 3.38 |
| 5.53 | 3.03 |
| 16.18 | 3.36 |
| 25.17 | 3.25 |
| 34.46 | 3.54 |

Fat At autopsy, a dull brown or chocolate brown liver was considered normal and a lighter brown liver with a yellow cast was considered perhaps to indicate fatty infiltration. However, the liver of guinea pig V S"A8 which had the lowest concentration of fat, was described as being brown with a yellow cast and yellow fringe while the liver of guinea pig VI S"B4, which had the highest concentration of fat was described as being reddish brown. The amounts of fat and the differences in concentrations of fat in the two livers were small; guinea pig V S"A8 had 2.12 per cent fat in its liver and guinea pig VI S"B4 had 4.72 per cent. Whereas hepatic color might be useful when concentrations of fat are more variable, the low concentrations and slight variations found in the present experiments precluded much change in the liver due to fat. The recorded colors were more likely to be associated with the completeness of bleeding and the activity of the liver insofar as bile production was concerned.

Except for small white spots observed on the surface of the liver of guinea pig V S"B5, a control animal, no gross abnormalities were seen.

Results of fat determinations for Experiments V and VI, which are given in Tables 26 and 27, indicated no alteration from control values in the concentration of fat in the liver of biotin-deficient guinea pigs. Only one guinea pig receiving avidin, V S"A8, had a fat concentration lower than the

Table 26. Concentration of moisture, nitrogen, and fat in livers of guinea pigs of Experiment V

| Animal | Liver weight (gm.) | Moisture | | Nitrogen | | Fat | |
|---------|--------------------|-------------|---------|-------------|---------|-------------|---------|
| | | Total (gm.) | Conc. % | Total (mg.) | Conc. % | Total (mg.) | Conc. % |
| V S"B1 | - | - | - | - | - | - | - |
| V S"B3 | 27.8 | 19.82 | 71.29 | 873 | 3.14 | 1073 | 3.86 |
| V S"B4 | 30.0 | 21.42 | 71.40 | 919 | 3.06 | 922 | 3.07 |
| V S"B5 | 21.0 | 14.95 | 71.19 | 729 | 3.47 | 790 | 3.76 |
| V S"B6 | 20.1 | 14.40 | 71.64 | 683 | 3.40 | 677 | 3.37 |
| V S"B7 | 24.3 | 17.16 | 70.62 | 799 | 3.29 | 1045 | 4.30 |
| Average | - | - | 71.23 | - | 3.27 | - | 3.67 |
| V S"A8 | 19.3 | 13.78 | 71.40 | 508 | 2.63 | 409 | 2.12 |
| V S"A9 | 20.8 | 14.86 | 71.44 | 679 | 3.26 | 846 | 4.07 |
| V S"A10 | 29.2 | 21.36 | 73.15 | 916 | 3.14 | 1175 | 4.02 |
| V S"A11 | 24.9 | 18.04 | 72.45 | 824 | 3.31 | 852 | 3.42 |
| V S"A12 | 23.8 | 17.54 | 73.70 | 777 | 3.26 | 980 | 4.12 |
| V S"A13 | 17.2 | 12.28 | 71.40 | 617 | 3.59 | 598 | 3.47 |
| V S"A14 | - | - | - | - | - | - | - |
| Average | - | - | 72.26 | - | 3.20 | - | 3.54 |
| V C15 | 21.5 | 15.68 | 72.93 | 657 | 3.06 | 720 | 3.35 |
| V C16 | 25.9 | 17.80 | 68.75 | 715 | 2.98 | 720 | 3.01 |
| V C17 | 15.5 | 11.16 | 72.00 | 532 | 3.43 | 614 | 3.96 |
| V C19 | 21.6 | 15.56 | 72.04 | 715 | 3.31 | 950 | 4.40 |
| V C20 | 22.6 | 16.32 | 72.21 | 706 | 3.12 | 905 | 4.00 |
| V C21 | 26.8 | 19.54 | 72.91 | 841 | 3.14 | 1083 | 4.04 |
| Average | - | - | 71.81 | - | 3.17 | - | 3.79 |
| V CA23 | 22.7 | 16.50 | 72.71 | 630 | 2.78 | 828 | 3.65 |
| V CA24 | 22.6 | 15.90 | 70.35 | 666 | 2.95 | 686 | 3.04 |
| V CA25 | 20.0 | 14.32 | 71.60 | 673 | 3.36 | 771 | 3.85 |
| V CA26 | 18.3 | 13.08 | 71.48 | 577 | 3.15 | 355 | 3.57 |
| V CA27 | 16.2 | 11.34 | 70.00 | 591 | 3.65 | 689 | 4.25 |
| V CA28 | 17.6 | 12.73 | 72.33 | 550 | 3.12 | 541 | 3.07 |
| Average | - | - | 71.41 | - | 3.17 | - | 3.57 |

Table 27. Concentration of moisture, nitrogen, and fat in livers of guinea pigs of Experiment VI

| Animal | Liver weight (gm.) | Moisture | | Nitrogen | | Fat | |
|-----------|--------------------|-------------|------------------------------|-------------|----------------|-------------|----------------|
| | | Total (gm.) | Conc. | Total (mg.) | Conc. % | Total (mg.) | Conc. % |
| VI S "B1 | 16.15 | 11.38 | 70.50 | 489 | 3.03 | 584 | 3.62 |
| VI S "B2 | 14.1 | 9.91 | 70.27 | 433 | 3.07 | 546 | 3.87 |
| VI S "B3 | 26.25 | 18.36 | 69.95 | 728 | 3.77 | 1088 | 4.15 |
| VI S "B4 | 11.50 | 8.08 | 69.63 | 396 | 3.44 | 542 | 4.72 |
| VI S "B6 | 13.8 | 9.72 | 70.46 | 457 | 3.31 | 523 | 3.79 |
| VI S "B7 | 10.0 | 6.96 | 69.62 | 347 | 3.47 | 363 | 3.63 |
| VI S "B8 | 13.2 | 9.16 | 69.36 | 477 | 3.61 | 526 | 3.98 |
| VI S "B9 | 18.25 | 12.93 | 70.85 | 577 | 3.16 | 728 | 3.99 |
| VI S "B10 | 11.6 | 8.04 | 69.33 | 413 | 3.56 | 465 | 4.01 |
| VI S "B11 | 15.85 | 11.34 | 71.53 | 510 | 3.22 | 530 | 3.34 |
| VI S "B12 | 15.45 | 10.85 | 70.24 | 492 | 3.18 | 603 | 3.90 |
| VI S "B13 | 16.50 | 11.69 | 70.86 | 483 | 2.92 | 579 | 3.49 |
| Average | - | - | 70.26 ±0.674 ^a | - | 3.31 ±0.260 | - | 3.87 ±0.357 |
| VI S "A14 | 10.0 | 6.89 | 68.94 | 347 | 3.47 | 423 | 4.23 |
| VI S "A15 | 10.7 | 7.53 | 70.40 | 320 | 2.99 | 373 | 3.48 |
| VI S "A16 | 6.5 | 4.58 | 69.83 | 231 | 3.55 | 223 | 3.43 |
| VI S "A17 | 7.9 | 5.51 | 69.71 | 272 | 3.44 | 250 | 3.17 |
| VI S "A18 | 11.3 | 8.01 | 70.87 | 389 | 3.44 | 474 | 4.19 |
| VI S "A21 | - | - | - | - | - | - | - |
| VI S "A22 | 5.55 | 3.76 | 70.36 | 200 | 3.74 | 167 | 3.12 |
| VI S "A24 | 7.2 | 4.94 | 68.64 | 336 | 4.66 | 278 | 3.86 |
| VI S "A25 | 8.3 | 5.65 | 68.04 | 245 | 2.95 | 334 | 4.03 |
| VI S "A26 | 6.45 | 4.38 | 67.83 | 296 | 4.58 | 279 | 4.33 |
| Average | - | - | 69.10 ±1.088 | - | 3.62 ±0.618 | - | 3.73 ±0.470 |

^aStandard deviation

controls and none of the guinea pigs receiving avidin had concentrations which were higher than the controls. The average fat concentrations for the groups receiving avidin were in each case similar to their control groups, the averages in

Experiment V being 3.67, 3.54, 3.79, and 3.57 for groups S"B, S"A, C, and CA, respectively, and in Experiment VI, 3.87 and 3.73 for groups S"B and S"A.

During the early evaluation of the role of biotin in nutrition, some fatty livers were attributed to biotin excess. Gavin and McHenry (1941) had reported that biotin produced fatty livers when fed to rats previously depleted of the B complex vitamins. Best et al. (1946) disputed these findings and attributed such fatty livers to a lack of labile methyl groups because their work indicated that fat accumulation could be prevented by addition of choline.

Because of the early implications of a relationship between biotin and oleic acid in bacteria (Williams and Fieger, 1946, and Williams et al., 1947), the question as to whether or not biotin is concerned with fat synthesis in higher animals has been investigated recently. At the present time limited information is available and different conclusions have been drawn; whether biotin is necessary for synthesis of fat in higher animals as it is for oleic acid synthesis in bacteria (Ravel and Shive, 1955) still remains to be determined. Okey et al. (1951) reported that incipient or borderline biotin deficiency in cholesterol-fed rats resulted in decreased accumulation of fat and cholesterol in their livers. Guggenheim and Olson (1952) found no difference in the rate of incorporation of C¹⁴ into hepatic fatty acids of

pair-fed and biotin-deficient rats. Earlier, Curran (1950) had reported a slight increase in the synthesis of hepatic fat during biotin deficiency on the basis of the amount of deuterium found in fat after deuterium oxide was given orally. Although the ratio of hepatic fatty acid to body weight was smaller in deficient rats, the concentration of deuterium in the fat isolated was higher than in control animals, suggesting that the rate of synthesis was increased. Curran attributed the increased rate of synthesis to inanition rather than to biotin deficiency, however, since earlier workers (Bernhard and Steinhauser, 1943) had found an increased rate of fat synthesis during inanition. Curran's findings do not agree with those of Gram and Okey (1958). They concluded that in biotin-deficient rats glyceride, phospholipid, and cholesterol synthesis in the liver was inhibited and C^{14} -labeled acetate was used preferentially for glycogen synthesis. Their conclusions were based on total hepatic fat and its specific activity rather than on the concentration of fat. Concentrations of fat in the livers of rats were 4.46, 5.50 and 4.75 per cent for biotin-deficient rat, pair-weighed, and ad libitum-fed, rats respectively, the total amounts of fat in the livers of these groups being 250, 333, and 415 mg. respectively with specific activities of 164, 653, and 159 counts per minute per milligram. The low specific activity for the ad libitum-fed group was considered to be due to the

readily available supply of acetate from food and endogenous sources.

Total amounts of fat, and concentrations, were determined in this study. As in Gram and Okey's study the concentrations of fat in the livers of biotin-deficient guinea pigs were similar to the concentrations of fat found in the livers of control guinea pigs, but the total amounts of fat were lower.

In inanition the amount of fat in the liver generally increases as long as there are endogenous stores of fat available to be mobilized to take care of energy needs (Peters and Van Slyke, 1946). Despite varying degrees of weight losses, 0.7 to 21.4 per cent of maximum body weights, there was no parallel increase in concentration of fat in biotin-deficient guinea pigs although at autopsy visible fat deposits were present.

Nitrogen Significant changes in hepatic nitrogen concentrations were not found in the present study (Tables 26 and 27). Also when hepatic nitrogen was calculated on a body weight basis for the guinea pigs of Experiment VI (Table 28) the averages for the two groups were similar.

Knowledge of the biochemical role of biotin in protein metabolism is limited to synthesis of aspartic acid (Stokes *et al.*, 1947) and the deamination of aspartic acid, serine, and threonine (Lichstein and Umbreit, 1947a) in bacteria.

Table 28. Relation of hepatic nitrogen to body weight for guinea pigs of Experiment VI

| Animal | Final body weight (gm.) | Total hepatic nitrogen (mg.) | Hepatic nitrogen per 100 gm. body wt. (mg.) |
|----------|-------------------------|------------------------------|---|
| VI S"B1 | 281 | 489 | 174 |
| VI S"B2 | 245 | 433 | 177 |
| VI S"B3 | 429 | 728 | 170 |
| VI S"B4 | 253 | 396 | 157 |
| VI S"B6 | 374 | 457 | 122 |
| VI S"B7 | 246 | 347 | 141 |
| VI S"B8 | 279 | 477 | 171 |
| VI S"B9 | 359 | 577 | 160 |
| VI S"B10 | 240 | 413 | 172 |
| VI S"B11 | 282 | 510 | 181 |
| VI S"B12 | 295 | 492 | 167 |
| VI S"B13 | 316 | 483 | 153 |
| Average | - | - | 162±16.92 ^a |
| VI S"A14 | 234 | 347 | 148 |
| VI S"A15 | 185 | 320 | 173 |
| VI S"A16 | 178 | 231 | 129 |
| VI S"A17 | 179 | 272 | 153 |
| VI S"A18 | 281 | 389 | 138 |
| VI S"A21 | 185 | - | - |
| VI S"A22 | 169 | 200 | 118 |
| VI S"A24 | 143 | 336 | 235 |
| VI S"A25 | 209 | 245 | 117 |
| VI S"A26 | 159 | 296 | 182 |
| Average | - | - | 155±49.10 |

^aStandard deviation

Limited data have prevented any conclusions about the relationship of biotin to protein metabolism in higher animals. Kritsman *et al.* (1953) reported that biotin-deficient rats receiving methionine-S³⁵ incorporated the label into proteins of different organs at a decreased rate. Administration of

10 mg. of biotin per day for 2 successive days prior to injection of methionine-S³⁵ resulted in a normal rate of inclusion. Biotin-deficient young rats incorporated less label than deficient adults. When tissues from biotin-deficient rats were studied in vitro addition of biotin to the substrate increased the uptake of labeled methionine.

Poznansksys (1957) found that serum albumin synthesis by liver slices and amylase synthesis by pancreatic slices were reduced in biotin-deficient chicks. Both reductions could be prevented by a single intraperitoneal injection of 100 mg. of biotin into the biotin-deficient chicks 24 hours prior to in vitro experiments. Synthesis of pancreatic amylase could also be raised to a value comparable to that found in the control chicks by the addition of α -ketoglutarate; some stimulation also was produced by fumarate. The addition of either α -ketoglutarate or fumarate to slices obtained from control chicks did not affect synthesis of pancreatic amylase. Glutamine, but not α -ketoglutarate, increased albumin synthesis to 70-80 per cent that occurring in liver slices of control animals. The α -ketoglutarate presumably was not effective because it could not pass through the cell membrane. Poznansksys concluded that biotin did not participate directly in protein synthesis, but in the synthesis of dicarboxylic acids of the citric acid cycle which were essential for oxidative phosphorylation which, in turn,

provided energy for protein synthesis.

Summary While biotin-deficient guinea pigs had lower liver weight-body weight ratios than their controls, their hepatic composition with respect to nitrogen, fat, and moisture remained similar to those of control guinea pigs in both Experiments V and VI. The effect of biotin deficiency, either directly or indirectly, as through inanition, was on the liver as a whole rather than on any of the major components, although biotin concentration itself was reduced in the biotin-deficient guinea pigs.

Kidney findings

The kidneys of the guinea pigs were examined in each experiment at autopsy for evidence of damage such as pitting, scarring, or hemorrhage. They were also weighed during the final experiment.

The gross appearance of kidneys changed only in the final experiment. When guinea pigs S"A16 and 24 were sacrificed early in Experiment VI, the cortices of their kidneys were not uniformly colored, but instead, lighter areas penetrated below the surface and appeared to be caused by infiltration of fat. Originally kidneys had been evaluated only by autopsy findings, but in view of the gross changes in the kidneys of S"A16 and S"A24, fat was determined in the kidneys of the remaining guinea pigs. Subsequently, renal cortical changes

were noted in two other guinea pigs, S"A18 and S"A21, but the kidneys of S"A21 were not saved for analysis because death had occurred several hours before autopsy. Thus, of these four guinea pigs, only the kidneys of guinea pig S"A18 were analyzed for fat.

While guinea pig S"A18 did have the highest concentration of renal fat of the experimental animals, higher values occurred in their controls (Table 29). The fat concentrations in the kidneys of the two groups were similar; averages for the biotin-deficient group and the control group were 3.71 and 4.04 per cent, respectively, medians were 4.08 and 3.78 per cent, and ranges were 2.67 - 4.40 and 3.31 - 5.48 per cent.

The kidney weight-body weight ratios were calculated in Experiment VI (Table 29). An average ratio of 0.0085 was found in the control group compared with 0.0118 for the biotin-deficient group. Reid and Briggs (1953) reported a value of 0.0090 grams for the kidney-body weight ratios of normally growing, 60-day old guinea pigs fed a synthetic diet, a value similar to the control value obtained here. When the two groups of Experiment VI were compared, calculating ratios on the basis of body weight minus gastrointestinal tract, the ratio for the control group was 0.0119 and for the experimental group, 0.0159.

According to Keys et al. (1950), the kidney undergoes atrophy in both acute and chronic starvation but the degree

Table 29. Weight of kidneys and concentration of fat in the kidneys of guinea pigs of Experiment V

| Animal | Wt. of kidneys (gm.) | Ration of kidney wt. to body wt. | Ratio of kidney wt. to body wt. minus GI tract | Renal fat | |
|-----------|----------------------|----------------------------------|--|-------------|--------------------------|
| | | | | Total (mg.) | Concentration (per cent) |
| VI S "B1 | 1.8 | 0.0064 | 0.0090 | 89 | 4.93 |
| VI S "B2 | 2.7 | 0.0110 | 0.0148 | 95 | 3.51 |
| VI S "B3 | 3.3 | 0.0077 | 0.0092 | 123 | 3.73 |
| VI S "B4 | 2.5 | 0.0099 | 0.0132 | - | - |
| VI S "B6 | 2.8 | 0.0075 | 0.0111 | 109 | 3.82 |
| VI S "B7 | 2.2 | 0.0089 | 0.0142 | - | - |
| VI S "B8 | 2.4 | 0.0086 | 0.0124 | 76 | 3.09 |
| VI S "B9 | 2.4 | 0.0067 | 0.0084 | 132 | 5.48 |
| VI S "B10 | 2.5 | 0.0104 | 0.0140 | 98 | 3.89 |
| VI S "B11 | 2.3 | 0.0082 | 0.0108 | 114 | 4.98 |
| VI S "B12 | 2.6 | 0.0088 | 0.0122 | 88 | 3.31 |
| VI S "B13 | 2.6 | 0.0082 | 0.0109 | 97 | 3.73 |
| Average | - | 0.0085 | 0.0119 | - | 4.04±0.72 ^a |
| VI S "A14 | 2.2 | 0.0094 (0.0092) ^b | 0.0117 | 94 | 4.29 |
| VI S "A15 | 2.3 | 0.0124 (0.0123) | 0.0161 | 94 | 4.08 |
| VI S "A16 | 2.7 | 0.0153 (0.0138) | 0.0193 | - | - |
| VI S "A17 | 1.4 | 0.0078 (0.0068) | 0.0128 | 48 | 3.44 |
| VI S "A18 | 2.5 | 0.0089 (0.0088) | 0.0112 | 110 | 4.40 |
| VI S "A21 | - | - | - | - | - |
| VI S "A22 | 2.0 | 0.0118 (0.0098) | 0.0169 | 52 | 2.67 |
| VI S "A24 | 2.5 | 0.0174 (0.0154) | 0.0225 | - | - |
| VI S "A25 | 2.2 | 0.0105 (0.0083) | 0.0145 | 93 | 4.24 |
| VI S "A26 | 2.0 | 0.0125 (0.0110) | 0.0182 | 57 | 2.84 |
| Average | - | 0.0118 (0.0106) | 0.0159 | - | 3.71±0.85 |

^aStandard deviation

^bKidney weight-body weight ratio based on maximum body weight

of renal loss is generally less than that of the body weight as a whole. When, in Experiment VI, the kidney-body weight ratios of the experimental group were recalculated on the basis of maximum body weight, an average value of 0.0106 was obtained. This ratio was still higher than that of the control group, 0.0085. However, this calculation lowered the high ratios found in the biotin-deficient group somewhat since some of the guinea pigs having the highest ratios also had the greatest weight losses; nevertheless, the ratios for guinea pigs S"Al6 and 24 remained higher than any for the controls.

No values for kidney-body weight ratios during biotin deficiency in any species were found. Katsh et al. (1955), who studied organ-body weight changes in biotin-deficient rats, did not include observations of the kidney. Pecora and Highman (1953) found increased kidney-body weight ratios in thiamine-deficient rats and to a lesser degree in their paired controls. The authors concluded, therefore, that in thiamine deficiency there was an increased kidney-body weight ratio which was in part due to the effect of inanition and in part due to a direct effect of thiamine deficiency. Pecora and Highman suggested that since kidney weight is determined mainly by the amount of work done by the kidney (Walter and Addis, 1939) the increase in kidney-body weight ratio occurring during thiamine deficiency must be explained in terms of

additional work being done by the kidney. They reasoned that the increase in kidney-body weight ratio observed in inanition was caused by increased work by the kidney of an animal losing weight and thus having a proportionally larger body surface area than its controls. McIntosh (1928) reported that urea clearance values, used in measuring renal function, were proportional to body surface area; hence, he concluded that the amount of work done by the kidney was also proportional to the surface area. Pecora and Highman suggested that the enlargement not caused by inanition in the thiamine-deficient rat might have been caused by the increased work performed by the kidney as it attempted to maintain ionic equilibrium while sodium was increasing and potassium decreasing in the tissues of thiamine-deficient rats.

The increased kidney-body weight ratios in the biotin-deficient guinea pigs might, in part, have been due to their proportionally larger body surface than the controls and the correspondingly increased kidney function, but it seemed unlikely that it could account entirely for the large kidney-body weight ratios of guinea pigs S["]A15, 16, and 24. The extent of the effects of inanition could not be estimated since there were no pair-fed controls. Although tissue analysis for sodium and potassium changes were not made in the present study, such changes might have occurred in biotin-deficient guinea pigs since their adrenals were reacting to stress.

In summary, biotin-deficient guinea pigs had increased kidney-body weight ratios which might have been caused by inanition and by other changes resulting in increased work for the kidney. Four biotin-deficient guinea pigs appeared to have an infiltration of fat and/or possibly the beginning of degenerative changes in the cortex of their kidneys.

Spleen findings

Spleens were weighed in Experiments V and VI. In Experiment V average spleen weight-body weight ratios of the groups receiving the synthetic diet, groups S"B and S"A, and the groups receiving the commercial ration, groups C and CA, were similar to those given by Reid and Briggs (1953) for groups receiving a synthetic diet and a commercial pelleted ration, their ratios being 0.00137 and 0.00113, respectively. The average ratios in Experiment V were 0.0016, 0.0016, 0.0010, and 0.0012 for the S"B, S"A, C, and CA groups (Table 30). In Experiment VI the values for groups S"B and S"A were somewhat higher than those for similar groups in Experiment V and than those reported by Reid and Briggs; this difference might be due to the fact that guinea pigs in Experiment VI were younger at autopsy than either those of Experiment V or those studied by Reid and Briggs. Eaton (1931) had reported previously that the spleen-body weight ratios decreased with age in growing guinea pigs. The ratio for the VI S"B group averaged 0.0019

Table 30. Weight of spleens of guinea pigs in Experiments V and VI

| Animal | Wt. of spleen (gm.) | Ratio of spleen wt. to body wt. | Animal | Wt. of spleen (gm.) | Ratio of spleen wt. to body wt. |
|---------|---------------------|---------------------------------|----------|---------------------|---------------------------------|
| V S"B1 | - | - | VI S"B1 | 0.52 | 0.0018 |
| V S"B2 | - | - | VI S"B2 | 0.48 | 0.0020 |
| V S"B3 | 0.89 | 0.0015 | VI S"B3 | 0.86 | 0.0020 |
| V S"B4 | 0.96 | 0.0017 | VI S"B4 | 0.32 | 0.0013 |
| V S"B5 | 0.86 | 0.0017 | VI S"B6 | 0.52 | 0.0014 |
| V S"B6 | 0.84 | 0.0015 | VI S"B7 | 0.35 | 0.0014 |
| V S"B7 | 0.83 | 0.0015 | VI S"B8 | 0.62 | 0.0022 |
| Average | | 0.0016 | VI S"B9 | 0.79 | 0.0022 |
| | | | VI S"B10 | 0.58 | 0.0024 |
| V S"A8 | 0.70 | 0.0018 | VI S"B11 | 0.72 | 0.0026 |
| V S"A9 | 0.89 | 0.0019 | VI S"B12 | 0.42 | 0.0014 |
| V S"A10 | 1.21 | 0.0020 | VI S"B13 | 0.62 | 0.0020 |
| V S"A11 | 0.83 | 0.0013 | Average | | 0.0019 |
| V S"A12 | 0.63 | 0.0011 | | | |
| V S"A13 | 0.68 | 0.0015 | VI S"A14 | 0.59 | 0.0025 |
| V S"A14 | - | - | VI S"A15 | 0.45 | 0.0024 |
| Average | | 0.0016 | VI S"A16 | 0.35 | 0.0020 |
| | | | VI S"A17 | 0.22 | 0.0012 |
| V C15 | 0.63 | 0.0010 | VI S"A18 | 0.38 | 0.0013 |
| V C16 | 0.74 | 0.0012 | VI S"A21 | - | - |
| V C17 | 0.50 | 0.0010 | VI S"A22 | 0.15 | 0.0009 |
| V C19 | 0.61 | 0.0010 | VI S"A24 | 0.17 | 0.0012 |
| V C20 | 0.75 | 0.0011 | VI S"A25 | 0.50 | 0.0024 |
| V C21 | 0.64 | 0.0010 | VI S"A26 | 0.24 | 0.0015 |
| Average | | 0.0010 | Average | | 0.0017 |
| | | | | | |
| V CA23 | 0.66 | 0.0011 | | | |
| V CA24 | 0.72 | 0.0011 | | | |
| V CA25 | 0.62 | 0.0012 | | | |
| V CA26 | 0.80 | 0.0014 | | | |
| V CA27 | 0.82 | 0.0014 | | | |
| V CA28 | 0.48 | 0.0009 | | | |
| Average | | 0.0012 | | | |

and the VI S"A group, 0.0017.

Both groups in Experiment VI had a wide range of spleen-body weight ratios, from 0.0013 to 0.0026 for the S"B group, and from 0.0009 to 0.0025 for the S"A group. These wide ranges were not due to different lengths of time on experiment; the smaller ratios were not found in guinea pigs on experiment longer nor the larger ratios in guinea pigs on experiment a shorter period of time. Also the variation in the ratios of the S"A group was not related to weight loss. Jackson (1925) had reported that the spleens of mature guinea pigs generally atrophied during conditions of inanition and malnutrition and when splenic enlargement was found it was mostly due to complications such as infections. Further, Jackson had reported that the relative loss in spleen weight was usually greater than loss in body weight, although the spleen lost weight slowly in early stages of inanition. In young guinea pigs results were even more variable than with mature guinea pigs; in some cases the weight of the spleen decreased while in others the inherent growth impulse appeared to overcome the tendency to atrophy during inanition. Similar spleen changes during inanition have been cited by Keys et al. (1950).

For biotin-deficient rats, Katsh et al. (1955) reported a spleen-body weight ratio of 0.0017 and for pair-fed control rats, 0.0015. Pecora and Highman (1953) found lower spleen-

body weight ratios in thiamine-deficient rats than in controls of the same age or in younger rats with similar body weights, but the ratios were the same as those of pair-fed controls. Pecora and Highman felt that part of the difference between spleen-body weight ratios of deficient and control rats might be attributed to decreased hematopoiesis occurring in the spleen of the deficient rats which needed less blood because they were losing weight.

Besides changes due to age and weight loss, spleen size might also change as part of a general adaptation syndrome. The spleen weight could decrease for two reasons; firstly, the volume of red blood cells is increased during shock presumably as a result of hemoconcentration and discharge into the circulation of red cells stored in the spleen and bone marrow, and secondly, the spleen may undergo involution along with other lymphatic tissues (Selye, 1947). The only guinea pig evidencing hemoconcentration and hemorrhagic adrenals was VI S"A22. It also had the lowest spleen-body weight ratio, 0.0009.

Adrenal findings

The size, appearance and cholesterol content of adrenal glands have been evaluated in these experiments because adrenal cortical activity is related to stress. Later different stages in the development of the adaptation syndrome

were related to adrenal changes (Selye and Stone, 1950). Sayers et al. (1944) have also related different types of stress to adrenal changes. Deficiencies of pantothenic acid and ascorbic acid have been intimately associated with changes in adrenal glands, the former possibly through the coenzyme A molecule necessary for cholesterol formation (McHenry, 1955) and the latter in some unknown way.

No changes in adrenal glands have been reported in most descriptions of biotin deficiency in other species. Terroine (1956) observed no changes in the histology or size of the adrenal gland in biotin-deficient rats, while Katsh et al. (1955) found increased adrenal size but no histological changes in both biotin-deficient rats and their pair-fed controls. Hartman and Brownell in their book The Adrenal Gland (1949) stated that every type of stress, if of sufficient magnitude or duration, could be shown to affect the adrenal cortex. Stresses of various kinds have increased the size of adrenals and dietary changes associated with such changes include inanition and deficiencies of vitamin A, thiamine, riboflavin, ascorbic acid, vitamin D, vitamin E, pantothenic acid, and nicotinic acid. With most of these deficiencies, whether the change is a primary one due to the deficiency or secondary due to generalized stress caused by the deficiency is not known.

Gross changes in adrenal glands did not occur, except in

one case, until the synthetic diet plus avidin was used to develop a deficiency. Of all animals fed the basal synthetic diet, only guinea pig III S"15 had hemorrhagic adrenal glands at autopsy. This guinea pig was very small at the beginning of the experiment; its weight pattern and hepatic biotin concentration were similar to the weight patterns and hepatic biotin concentrations of deficient guinea pigs in Experiment VI. The one guinea pig (S"A5) receiving synthetic diet plus avidin in Experiment IV had slightly hemorrhagic adrenal glands and four (S"A21, 22, 25, and 26) of 10 guinea pigs receiving synthetic diet plus avidin in Experiment VI had hemorrhagic adrenal glands. In each instance when adrenal hemorrhages occurred, indications of biotin deficiency such as lowered hepatic biotin, unthrifty appearance and slow growth or weight loss were present, but severe external symptoms such as those reported in biotin-deficient rats never developed. Guinea pigs I^V S"A5, VI S"A25, and VI S"A26 were autopsied when their physical conditions indicated they were unlikely to live much longer; guinea pig VI S"A22 became prostrate on the 32nd day of the experiment and was autopsied immediately; and VI S"A21 was found dead the morning of the 33rd day.

In the first three experiments only an estimation of the relative size of the adrenal glands was made while in the three subsequent experiments they were weighed. The weights

of the adrenal gland were calculated as milligrams per 100 grams body weight and milligrams per 100 grams body weight minus gastrointestinal tract (Tables 31, 32, and 33). Weights were compared in this manner because of the wide variation in age and weight of the guinea pigs when they were sacrificed. A comparison made in this manner might err slightly because

Table 31. Weight of adrenal glands of guinea pigs, Experiment IV

| Animal | Adrenal weight | | |
|----------|--------------------|-----------------------------|--|
| | Mg. | Mg. per 100 gm. body weight | Mg. per 100 gm. body weight minus GI tract |
| IV S#1 | 172.0 | 48.4 | 60.1 |
| IV S#4 | 156.0 | 141.8 (78.3) ^a | 175.3 |
| IV S#A5 | 196.0 ^b | 95.1 (72.6) ^a | - |
| IV S#B7 | 90.5 | 52.0 | 79.4 |
| IV S#B8 | - | - | - |
| IV S#BA9 | 111.0 | 39.6 | 56.6 |
| IV C10 | 116.7 | 45.6 | 63.2 |
| IV C11 | 166.0 | 37.4 | 44.7 |
| IV C12 | 30.6 | 27.5 | 35.5 |
| IV CA13 | 136.0 | 36.7 | 43.9 |

^aBased on maximum body weight

^bHemorrhagic

Table 32. Weight of adrenal glands of guinea pigs,
Experiment V

| Animal | Adrenal weight | | |
|---------|----------------|-----------------------------------|--|
| | Mg. | Mg. per 100 gm. body weight | Mg. per 100 gm. body weight minus GI tract |
| V S"B1 | - | - | - |
| V S"B3 | 306.4 | 53.3 | 64.0 |
| V S"B4 | 302.6 | 52.9 | 62.3 |
| V S"B5 | 248.6 | 48.3 | 57.2 |
| V S"B6 | 341.0 | 60.0 | 70.0 |
| V S"B7 | 272.0 | 50.6 | 62.7 |
| Average | - | 53.0 | 63.2 |
| V S"A8 | 156.5 | 40.1 | 54.7 |
| V S"A9 | 191.7 | 40.7 | 47.8 |
| V S"A10 | 273.8 | 44.2 | 51.9 |
| V S"A11 | 269.4 | 42.0 | 50.0 |
| V S"A12 | 269.8 | 46.2 | 55.2 |
| V S"A13 | 140.4 | 31.2 | 40.3 |
| V S"A14 | 221.0 | 63.9 ^a | - |
| Average | - | 44.0 | 49.9 |
| V C15 | 245.9 | 40.0 | 49.0 |
| V C16 | 175.0 | 27.5 | 33.0 |
| V C17 | 211.2 | 42.2 | 52.2 |
| V C19 | 227.4 | 38.2 | 46.8 |
| V C20 | 280.0 | 44.0 | 52.7 |
| V C21 | 191.7 | 28.5 | 33.9 |
| Average | - | 36.7 | 44.6 |
| V CA23 | 145.8 | 25.2 | 30.5 |
| V CA24 | 191.6 | 30.5 | 38.3 |
| V CA25 | 199.0 | 37.9 | 46.9 |
| V CA26 | 198.0 | 33.8 | 39.9 |
| V CA27 | 238.6 | 40.6 | 48.3 |
| V CA28 | 106.4 | 20.7 | 24.7 |
| Average | - | 31.4 | 38.1 |

^aRemoved after death, not included in average

Table 33. Weight of adrenal glands of guinea pigs, Experiment VI

| Animal | Mg. | Adrenal weight | |
|----------|--------------------|-----------------------------|--|
| | | Mg. per 100 gm. body weight | Mg. per 100 gm. body weight minus GI tract |
| VI S"B1 | 163.8 | 58.3 | 81.5 |
| VI S"B2 | 147.4 | 60.2 | 81.1 |
| VI S"B3 | 156.0 | 36.4 | 43.6 |
| VI S"B4 | 110.8 | 44.8 | 58.6 |
| VI S"B6 | 146.4 | 39.1 | 58.1 |
| VI S"B7 | 113.5 | 46.1 | 73.2 |
| VI S"B8 | 99.7 | 35.7 | 51.6 |
| VI S"B9 | 157.8 | 44.0 | 55.8 |
| VI S"B10 | 135.8 | 55.6 | 75.8 |
| VI S"B11 | 139.8 | 49.6 | 65.6 |
| VI S"B12 | 146.0 | 49.5 | 68.5 |
| VI S"B13 | 164.6 | 52.1 | 69.2 |
| Average | - | 47.7 \pm 8.2 ^a | 65.2 |
| VI S"A14 | 131.2 | 57.4 (55.9) ^b | 71.4 |
| VI S"A15 | 137.8 | 74.5 (73.7) | 96.4 |
| VI S"A16 | 121.5 | 69.0 (62.0) | 86.4 |
| VI S"A17 | 162.1 | 90.6 (79.6) | 148.7 |
| VI S"A18 | 152.2 | 54.2 (53.8) | 68.3 |
| VI S"A21 | - ^c | - | - |
| VI S"A22 | 204.0 ^c | 120.7 (99.5) | 172.8 |
| VI S"A24 | 120.0 | 83.9 (74.1) | 108.1 |
| VI S"A25 | 176.2 ^c | 84.3 (66.2) | 115.9 |
| VI S"A26 | 132.8 ^c | 79.5 (70.9) | 120.7 |
| Average | - | 79.5 (70.9) \pm 19.8 | 109.8 |

^aStandard deviation^bBased on maximum body weight^cHemorrhagic

Bessesen and Carlson (1923) found that up to a body weight of about 400 grams the curve for the adrenal gland-body weight ratio was slightly convex upward. They reported, however, that the adrenal glands were equivalent to approximately 0.05 per cent of the body weight of the guinea pig under 500 grams and there was an increase in the ratio in guinea pigs with weights over 500 grams.

When adrenal weights, expressed in milligrams per 100 grams of body weight or milligrams per 100 grams of body weight minus gastrointestinal tract, were compared, high values were found for two guinea pigs in Experiment IV (Table 31). The guinea pigs were IV S"4, which lost weight for about a week prior to its collapse and autopsy and IV S"A5, which was unkempt in appearance and lost weight prior to autopsy. Guinea pig IV S"4 did not have a reduced concentration of hepatic biotin, but guinea pig IV S"5 did. The weights of adrenal glands per 100 grams of body weight of guinea pig IV S"4 and IV S"A5 were similar to those of the VI S"A group when calculated on the basis of maximum weight. The values so calculated were 78.3 and 72.6 for guinea pigs IV S"4 and IV S"A5, respectively, and the average for the VI S"A group was 70.9.

In Experiments IV and V, the adrenal glands of guinea pigs receiving commercial rabbit pellets were smaller in proportion to body weight than those receiving synthetic diet

(Tables 31 and 32). Weights of adrenal glands per 100 grams of body weight were slightly higher but not significantly different for guinea pigs receiving synthetic diet plus biotin in Experiments IV, V, and VI (Tables 31, 32, and 33) than those reported by Reid and Briggs (1953) for guinea pigs receiving a complete synthetic diet, while values for guinea pigs in Experiments IV and V receiving pellets were lower than those reported by Reid and Briggs for guinea pigs receiving a similar ration. Reid and Briggs observed that adrenal glands weighed 46 ± 2 mg. per 100 grams of body weight irrespective of diet, whereas in this study the value for guinea pig IV S"B7 was 52.0 mg. and the averages for the S"B groups were 53.0 mg. for Experiment V and 47.7 mg. for Experiment VI. The average adrenal weights per 100 grams of body weight for groups receiving commercial rabbit pellets were 36.8 and 36.7 mg. for Experiments IV and V, respectively.

Possible biotin deficiencies were detected in only two guinea pigs, S"A8 and S"A14, in Experiment V. The adrenal weight of S"A8 was below the average of the S"A group when calculated per 100 grams of body weight, while the adrenal weight of S"A14 calculated in this manner was highest in the S"A group and in Experiment V.

In Experiment VI the adrenal weights were much higher in the biotin-deficient group than in the controls whether they were calculated per 100 grams of final body or maximum

body weight (Table 33). The average adrenal weight per 100 grams of final body weight for the control group was 47.7 milligrams and 79.8 for the biotin-deficient group. On the basis of maximum weight the latter figure was 70.9 mg. The difference between groups was similar when adrenal weight was calculated per 100 grams body weight minus gastrointestinal tract, the average values being 65.2 mg. for the S"B group and 109.8 mg. for the S"A group.

Since the absolute weights of adrenal glands of the two groups in Experiment VI were essentially the same with the exception of guinea pig S"A22, it was interesting to speculate on the relationship between adrenal weight and body weight during a deficiency when growth is retarded or weight lost. Oleson and Bloor (1941) reported that absolute weights of adrenal glands in mature guinea pigs did not change although considerable body weight was lost when food was withheld for either 3 to 7 days or for 10 to 14 days, at which time they were on the verge of death. However, both free and esterified cholesterol in the adrenal glands decreased. Oleson and Bloor interpreted this finding as an indication that the glands were probably protected from emaciation owing to their activity as vital organs. Blumenthal and Loeb (1942) found increased mitotic activity and increased cell size in the adrenal cortex of guinea pigs during moderate inanition, but if inanition was either severe or slight, mitotic activity was suppressed.

Whereas size may be an indication of activity of adrenal glands an increase in size may also be caused by congestion or edema (Hartman and Brownell, 1949). Adrenal cholesterol determinations have been made as a means of assessing the activity of the gland since Haines et al. (1951) demonstrated that cholesterol was an intermediate in hormone synthesis in the adrenal gland. The determinations have been limited in their usefulness since they indicated the status of the adrenal glands only at the time animals were sacrificed and gave no indication whether their cholesterol content was increasing, decreasing, or being maintained.

Sayers et al. (1944) have formulated five types of response of the adrenal cortex to alterations in external or internal environment and have related these responses to alterations in size and cholesterol content. The type of response is determined to a large degree by the severity and duration of the stress. The first three types are of interest in interpreting adrenal cholesterol and adrenal weight changes in the present experiments. Type I is caused by moderate stress applied to the animal for a relatively short period of time. It is characterized by reduced concentrations of adrenal cholesterol and sudanophilic material within a few hours after application of stress. When the stress is removed the concentrations of adrenal cholesterol and sudanophilic material slowly return to their initial values. Under

these conditions there may be slight or no hypertrophy of the gland.

The Type II response is obtained when stress caused by external or internal environment proceeds at a slow rate. Since there is a gradual increase in the demand for cortical hormones, the concentration of adrenal cholesterol remains practically normal, but the actual mass of glandular tissue increases so that the increased demand for the hormone is met, not by a sudden depletion as in Type I, but by an increase in the number of functional units. Moderate fasting (calories reduced to one-half of ad libitum intake), unless it is extended to the point of collapse of the animal, gives a Type II response. Although Sayers and coworkers (1944) did not mention chronic vitamin deficiencies, their similarity to restricted food intake leads one to expect a Type II response.

The Type III response develops when an animal is subjected to severe or sudden stress that is beyond its ultimate capacity to meet. The demand for the cortical hormone is not only excessive but it is continuous until death. During the period of survival, adrenal cholesterol and sudanophilic material are rapidly and markedly depleted. The adrenal gland may also hypertrophy, but the degree is determined by the interval between the time of the first appearance of the stress and the death. The animals frequently have enlarged

adrenal cortices which are devoid of cholesterol and lipids. Type III is produced by starvation and by severe vitamin deficiencies. In vitamin C deficiency in guinea pigs, cholesterol concentrations of the adrenal glands decreased from 5.1 per cent on a complete synthetic diet to 2.8 per cent after 26 to 32 days on a diet deficient in vitamin C. Decreases in adrenal cholesterol concentrations in other vitamin deficiencies were also cited by Sayers and coworkers (1944).

Responses of Types IV and V do not result as a consequence of the demand for an increased secretion of adrenal cortical hormone, but are associated with alterations in anterior lobe function.

The general adaptation syndrome as described by Selye (1937) is divided into three stages. The changes which occur during the first stage, or stage of alarm, appear 6 to 48 hours after the initial injury. During this stage there is a rapid decrease in the size of the thymus, spleen, lymph glands, and liver; fat tissue disappears; edema forms, especially in the thymus and loose retroperitoneal connective tissue; pleural and peritoneal transudates accumulate; muscle tone is lost; body temperature falls; acute erosions form in the digestive tract; cortical lipoids and chromaffin substance are lost from the adrenals; and sometimes hyperemia of the skin, exophthalmos, increased lachrymation, and saliva-

tion occur. If the injury or stimulation is continued for a period of hours or longer and the stress is not too severe, the organs return to a more normal condition, and the animal is in the second or resistant stage. During this time there is a reappearance of cortical lipoids. If the injury is continued still longer the animals can no longer respond and in the third stage, or stage of exhaustion, the animal dies with organ changes similar to those seen in the first stage. Selye and Stone (1950) have related these stages of the adaptation syndrome to changes which occur in the adrenal gland. During the alarm reaction the demand for cortical hormones is excessive. The adrenal cortex undergoes hypertrophy and hyperplasia; it increases in size, while discharging lipid stores into the blood. In the resistant stage the adrenal cortex is enlarged and its cells store numerous sudanophilic lipid granules, while in the stage of exhaustion there is a depletion of lipids.

Responses of Types I, II, and III as proposed by Sayer are not the same as the stages of alarm, resistance and exhaustion as proposed by Selye although certain features are similar. The stages succeed each other in response to one stress while the various types are based upon changes produced in the gland by different stresses. Both concepts, however, were useful in evaluating adrenal glands in Experiment VI.

Of guinea pigs in the experimental group, VI S"A14 and

18 could be classified as having only slight changes in their adrenal glands although low hepatic biotin concentrations and an unthrifty appearance plus loss of hair and hair color of guinea pigs S"A18 indicated that deficiencies existed. Their adrenal weights when calculated per 100 grams of body weight were similar to those in the upper range of values observed in control animals. VI S"A14 and 18 appeared to have a Type I response, or in terms of Selye's general adaptation syndrome, to be in the resistant stage. Their cyclic weight gains and losses indicated that although the deficiency was producing a stress the guinea pigs were adjusting to smaller amounts of biotin. On the basis of adrenal cholesterol concentrations (Table 34) and adrenal weights when calculated per 100 grams (Table 33), guinea pigs VI S"A14 and 18 were considered to have a moderate chronic deficiency.

On the basis of per cent adrenal cholesterol (Table 34) and adrenal gland-body weight ratios (Table 33) guinea pigs VI S"A15, 16, and 17 could be classified as having a Type II response, increased functioning tissue. Their adrenal weights when calculated per 100 grams of body weight were 74.5, 69.0, and 90.6, mg., considerably higher than the average for the controls, 47.7 mg. In terms of Selye's adaptation syndrome these three guinea pigs were in the stage of resistance and VI S"A17 was approaching the stage of exhaustion.

Table 34. Adrenal cholesterol concentrations of guinea pigs of Experiment VI

| Animal | Adrenal weight (mg.) | Total adrenal cholesterol (mg.) | Per cent adrenal cholesterol (%) | Final weight (gm.) | Body wt. minus GI tract (gm.) | Wt. of adrenal cholesterol per 100 gm. body wt. (mg.) | Wt. of adrenal cholesterol per 100 gm. body wt. minus GI tract (mg.) |
|-----------|----------------------|---------------------------------|----------------------------------|-----------------------|-------------------------------|---|--|
| VI S "B1 | 163.8 | 7.442 | 4.54 | 281 (46) ^a | 201 | 2.65 | 3.70 |
| VI S "B2 | 147.4 | 8.874 | 6.02 | 245 (35) | 183 | 3.62 | 4.85 |
| VI S "B3 | 156.0 | 8.841 | 5.67 | 429 (50) | 358 | 2.06 | 2.47 |
| VI S "B4 | 110.8 | 7.284 | 6.57 | 253 (25) | 189 | 2.88 | 3.85 |
| VI S "B6 | 146.4 | 6.742 | 4.60 | 374 (47) | 252 | 1.80 | 2.68 |
| VI S "B7 | 113.5 | 5.078 | 4.47 | 246 (23) | 155 | 2.06 | 3.28 |
| VI S "B8 | 99.7 | 8.225 | 8.25 | 279 (32) | 193 | 2.95 | 4.26 |
| VI S "B9 | 157.8 | 10.773 | 6.83 | 359 (46) | 283 | 3.00 | 3.81 |
| VI S "B10 | 135.8 | 7.251 | 5.34 | 240 (33) | 179 | 3.02 | 4.05 |
| VI S "B11 | 139.8 | 9.008 | 6.44 | 282 (35) | 213 | 3.19 | 4.23 |
| VI S "B12 | 146.0 | 7.043 | 4.82 | 295 (35) | 213 | 2.38 | 2.31 |
| VI S "B13 | 164.6 | 10.514 | 6.39 | 316 (47) | 238 | 3.35 | 4.42 |
| VI S "A14 | 134.2 | 8.417 | 6.27 | 234 (46) | 188 | 3.60 | 4.48 |
| VI S "A15 | 137.8 | 8.009 | 5.81 | 185 (33) | 143 | 4.33 | 5.60 |
| VI S "A16 | 121.5 | 8.317 | 6.90 | 176 (23) | 140 | 4.72 | 5.94 |
| VI S "A17 | 163.1 | 5.611 | 3.46 | 179 (35) | 109 | 3.14 | 5.15 |
| VI S "A18 | 152.2 ^b | 9.216 | 6.06 | 281 (49) | 223 | 3.28 | 4.13 |
| VI S "A21 | - | - | - | - | - | - | - |
| VI S "A22 | 204.0 ^b | 1.898 | 0.93 | 169 (31) | 118 | 1.12 | 1.61 |
| VI S "A24 | 120.0 | - | - | 143 (20) | 111 | - | - |
| VI S "A25 | 176.2 ^b | 10.919 | 6.20 | 209 (33) | 152 | 5.22 | 7.18 |
| VI S "A26 | 132.8 ^b | 5.295 | 3.99 | 159 (32) | 110 | 3.33 | 4.81 |

^aDays on experiment^bHemorrhagic adrenals

Two limitations are important in interpretation of cholesterol data; first, cholesterol can be rapidly depleted or repleted, and second, any enlargement of the gland to meet increased needs must be considered when concentrations are compared.

If total adrenal cholesterol is considered indicative of the hormone available to the body (Sayers et al., 1944) and is calculated on a body weight basis, the amount available to the tissues was higher in the experimental group than in the control group while the average concentrations in the glands were 4.95 per cent and 5.85 per cent, respectively (Table 34). The biotin-deficient group averaged 3.59 and 4.86 mg. adrenal cholesterol per 100 grams body weight and 100 grams body weight minus gastrointestinal tract, respectively, compared with 2.75 and 3.66 mg. for the control group. Table 34 shows the wide variation found in the weight of adrenal cholesterol per 100 grams of body weight due to different stages of stress produced in biotin-deficient guinea pigs.

Four guinea pigs in the experimental group had hemorrhagic adrenals. Guinea pig VI S"A24 died during the night so no cholesterol analysis was made since only tissues taken from live animals were used. Guinea pig VI S"A22 had the lowest adrenal cholesterol concentration, 0.93 per cent, and the lowest weight of adrenal cholesterol per 100 grams of body weight, 1.12 mg. Guinea pigs VI S"A25 and 26, autopsied

when collapse seemed imminent, also had hemorrhagic adrenals. Their adrenal cholesterol concentrations were 6.20 and 3.99 per cent. Although the adrenal glands were not severely depleted, this perhaps would have occurred if the guinea pigs had been allowed to collapse before autopsy. The adrenal glands of both animals were large in proportion to their body weight. These four guinea pigs were considered to have a Type III response, or be in the stage of exhaustion.

Changes in adrenal glands may account for certain other changes observed in biotin-deficient guinea pigs. Adrenal changes may have caused changes in the kidneys and spleen, renal changes as a result of its attempt to maintain ionic equilibrium in the body and splenic changes as a result of atrophy of lymphoid tissue and hemoconcentration. Selye (1937) mentioned that small livers were part of the adaptation syndrome and smaller livers, in terms of absolute weights or per cents of body weight, were found in the biotin-deficient guinea pigs than in the controls. Two other responses mentioned by Selye were noted at autopsy of deficient guinea pigs, fluid accumulation in the peritoneal cavity and small fat stores. It was impossible in Experiment VI to distinguish between adrenal changes due to inanition and due to response to stress of biotin deficiency since inanition itself can be considered a stress.

Organ weight-body weight changes

In the previous sections in which liver, spleen, kidneys, and adrenal glands of the biotin-deficient guinea pigs were compared with their controls, certain changes in the organ weight-body weight ratios were noted. Table 35 gives a summary of the average organ weight per 100 grams of body weight for the two groups in Experiment VI. The average hepatic weight was smaller in the biotin-deficient group than in the control groups and the average renal and adrenal weights were larger in the biotin-deficient group than in the control group. The average spleen weight in relation to body weight was not affected by biotin deficiency. Pecora and Highman (1953) found that livers and spleens were smaller in proportion to body weight whereas kidneys and adrenal glands were larger in proportion to body weight in thiamine-deficient animals than in their controls. Similar changes in organ weights have been observed as a result of reduced caloric intake (Jackson, 1925). Thus this pattern of organ weight changes found in the biotin-deficient group resembles those observed in thiamine and caloric deficiencies and is not specific for biotin deficiency.

Table 35. Average weight of organs per 100 grams final body weight and per 100 grams final body weight minus gastrointestinal tract for two groups of guinea pigs in Experiment VI

| Group | Liver | | Adrenal | |
|--------|------------------------|-------------------------------------|--------------------|-------------------------------------|
| | Final weight (gm.) | Minus gastro-intestinal tract (gm.) | Final weight (mg.) | Minus gastro-intestinal tract (mg.) |
| VI S"B | 5.05±0.72 ^a | 6.80±0.72 | 47.7±8.2 | 65.2±11.9 |
| VI S"A | 4.29±0.76 | 5.79±1.07 | 79.5±19.8 | 109.8±34.6 |

| Group | Kidney | | Spleen | |
|--------|--------------------|-------------------------------------|--------------------|-------------------------------------|
| | Final weight (gm.) | Minus gastro-intestinal tract (gm.) | Final weight (mg.) | Minus gastro-intestinal tract (mg.) |
| VI S"B | 0.85±0.14 | 1.19±0.22 | 0.19±0.04 | 0.28±0.02 |
| VI S"A | 1.18±0.31 | 1.59±0.38 | 0.17±0.07 | 0.26±0.02 |

^aStandard deviation

SUMMARY AND CONCLUSIONS

Six experiments have been performed with two proposed objectives: to produce biotin deficiency in guinea pigs without using egg white in the ration and to detect metabolic changes occurring as a result of the deficiency.

The first four experiments established performance records for guinea pigs under conditions in this laboratory and eliminated some of the possible methods for producing biotin deficiency. The use of aureomycin as a bacteriostatic agent to prevent intestinal synthesis of biotin was unsatisfactory since guinea pigs developed diarrhea and became shabby in appearance although their diet contained biotin. Addition of either sulfasuxidine or dl-desthiobiotin to a synthetic diet did not affect growth rate or produce other evidence of biotin deficiency. Guinea pigs receiving a synthetic diet lacking in biotin did not develop any signs of deficiency when 12 to 14 day-old guinea pigs were used, but when 3 to 7 day-old animals were used, two of six animals receiving the diet became unthrifty in appearance, gained weight slowly and decreased the efficiency with which they utilized food for growth. However, only one of these guinea pigs had a reduced concentration of hepatic biotin. One 3 to 7 day-old guinea pig received synthetic diet plus an avidin concentrate. It also had an unthrifty appearance, poor food

efficiency, slow weight gain, reduced hepatic biotin concentration, and in addition hemorrhagic adrenal glands. Guinea pigs receiving avidin as well as a dietary supply of biotin gained weight more slowly than their controls, but otherwise appeared to be healthy. From these results, biotin deficiency appeared to be produced most satisfactorily by feeding an avidin concentrate to guinea pigs consuming a synthetic diet which contained no biotin.

In the fifth experiment, an avidin concentrate was fed either with a synthetic diet or with commercial rabbit pellets to guinea pigs 12 to 15 days old. From the group receiving synthetic diet and avidin concentrate, one guinea pig died after a period of reduced weight gain and another had a reduced rate of growth, unkempt appearance, and a reduced concentration of hepatic biotin. Other guinea pigs in this group and guinea pigs receiving commercial rabbit pellets and avidin concentrate were unaffected by the dietary modifications. Their growth rates, hemoglobin concentrations, red blood cell volumes, and hepatic biotin concentrations were similar to their controls.

In the sixth experiment, when larger amounts of avidin concentrate were fed to younger guinea pigs (3 to 7 days old) than those used in Experiment V, biotin deficiency of varying severity was produced in all guinea pigs. The first symptoms were reduced weight gains and reduced food efficiencies.

Average daily weight gains ranged from 3.1 to 5.6 grams for the control guinea pigs and from 0.0 to 2.5 for the biotin-deficient guinea pigs; food efficiencies ranged from 0.22 to 0.46 grams of weight gained per gram of food eaten for the control guinea pigs and from 0.00 to 0.22 for the biotin-deficient guinea pigs. Six of the 10 biotin-deficient guinea pigs achieved maximum weight from 18 to 23 days after the experiment began; thereafter their weights declined. At 18 to 23 days, the average weight of control guinea pigs was 10 to 50 grams higher than weights of biotin-deficient guinea pigs.

As the sixth experiment progressed, the guinea pigs developed an unthrifty appearance which was a combination of gauntness and cottony fur. The gross manifestations of biotin deficiency were not as severe or as varied as gross symptoms reported for biotin-deficient rats. Symptoms such as spectacled eye and kangaroo gait did not occur. Dermatitis appeared in several instances, but was slight and did not persist. Biotin-deficient guinea pigs had thinner hair than their controls, but only two guinea pigs had definite nude regions. One guinea pig lost some of its hair color, but since a large number of the guinea pigs were albinos, possible effects of biotin deficiency on hair color could not be evaluated. As deficiencies became severe, guinea pigs frequently collapsed and died. Animals which collapsed prior to

autopsy did not differ in gross appearance from those sacrificed before collapse.

Biotin-deficient guinea pigs had reduced concentrations of biotin in their livers. The concentration of hepatic biotin associated with the symptoms of moderate to severe biotin deficiency in guinea pigs was 0.32 mcg. per gram of liver; livers of control guinea pigs averaged 0.59 mcg. biotin per gram.

Hemoglobin concentrations and red blood cell volumes of biotin-deficient animals were similar to their controls, although two guinea pigs of the deficient group had very low hemoglobin concentrations and red blood cell volumes. A third biotin-deficient guinea pig had a high hemoglobin concentration which may have been hemoconcentration as a result of adrenal changes.

Concentrations of nonprotein nitrogen in the blood of biotin-deficient guinea pigs varied widely as did concentrations of their controls, but with one exception all were within the normal range compiled by Spector (1956). Although concentrations of blood urea in both the control and the biotin-deficient groups also varied widely, they too were within the normal range.

The guinea pig which had a high nonprotein nitrogen concentration in its blood was one of three guinea pigs in the avidin-fed group that appeared to have an infiltration of

fat and/or degenerative changes in the renal cortex. However, since two other guinea pigs having similar changes in the kidneys had normal nonprotein nitrogen values, this renal change was not necessarily related to the instance of increased nonprotein nitrogen concentration. Renal fat concentrations in the biotin-deficient guinea pigs were not elevated above the concentrations of their controls.

Although concentrations of hepatic nitrogen, fat, and moisture were unaffected by biotin deficiency, liver size was reduced both in absolute weight and in proportion to body weight.

Four of 10 biotin-deficient guinea pigs of Experiment VI had hemorrhagic adrenal glands. The adrenal glands of biotin-deficient guinea pigs were larger than those of their controls whether expressed in terms of final body weight or maximum body weight. These changes in size plus variable changes in adrenal cholesterol concentrations of biotin-deficient guinea pigs indicated that guinea pigs were in different stages of the adaptative syndrome as a result of the stress of the deficiency.

Certain symptoms observed in biotin-deficient guinea pigs might have been secondary to adrenal changes, and failure to obtain symptoms observed in other species may be due to the relatively acute condition which developed in a short time in the experiments reported here and which lead

to death as a result of adrenal failure. Secondary changes included an increase in the renal weight-body weight ratio and a decrease in hepatic weight-body weight ratio. One guinea pig, which had hemorrhagic adrenals and hemoconcentration, also had a small spleen weight-body weight ratio, but other biotin-deficient guinea pigs showed no decrease in the spleen weight-body weight ratio.

Changes in the adrenal, hepatic, and renal weight-body weight ratios observed in the biotin-deficient guinea pigs may have resulted from caloric insufficiency. Similar changes have been reported for other deficiencies and hence are not specific for biotin deficiency.

In conclusion, biotin deficiency was produced in guinea pigs. It was obtained either by feeding a biotin-deficient synthetic diet to guinea pigs 3 to 7 days old or by feeding an avidin concentrate to guinea pigs receiving a biotin-deficient synthetic diet. Their growth rate was reduced, food efficiency impaired, and hepatic biotin lowered by the deficiency.

Changes associated with biotin deficiency which were noted at autopsy included hemorrhagic adrenals, smaller fat deposits than their controls, and certain differences in organ weight-body weight ratios. Organ weight-body weight changes noted may have been due to caloric insufficiency and/or adrenal changes, however, and are not specific for biotin deficiency.

Results of blood urea and nonprotein nitrogen analyses and of hepatic fat, nitrogen, and moisture analyses failed to indicate metabolic changes which might have affected their concentrations. Hemoglobin concentrations and red blood cell volumes of the biotin-deficient guinea pig also were not changed. Changes in intermediary metabolism of carbohydrates might have accounted for some of the symptoms observed, but no attempt was made to assess possible alterations of the various pathways for utilizing this source of energy.

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APPENDIX

Table 36. Per cent of intact body weight contributed by gastrointestinal tract

| Animal | Final weight (gm.) | Weight of gastrointestinal tract (gm.) | Weight due to gastrointestinal tract (%) |
|----------|--------------------|--|--|
| II S'B1 | 464 | 58.0 | 13.0 |
| II S'B5 | 298 | 82.9 | 27.8 |
| II S'B14 | 320 | 55.1 | 17.2 |
| II S'B17 | 501 | 129.1 | 25.8 |
| II S'2 | 425 | 116.5 | 27.4 |
| II S'9 | 304 | 64.5 | 21.2 |
| II S'18 | 506 | 97.3 | 19.2 |
| II S'21 | 537 | 101.0 | 18.8 |
| II S'D3 | 562 | 112.7 | 20.0 |
| II S'D10 | 449 | 78.4 | 17.5 |
| II S'D16 | 486 | 88.1 | 18.1 |
| II S'S4 | 400 | 63.3 | 15.8 |
| II S'S13 | 427 | 80.2 | 18.8 |
| II S'S22 | 437 | 113.5 | 30.0 |
| II S'S23 | 440 | 65.3 | 14.8 |
| III S"B1 | 352 | 68.4 | 19.4 |
| III S"B2 | 423 | 71.1 | 16.8 |
| III S"B5 | 297 | 79.0 | 26.6 |
| III C8 | 352 | 59.5 | 16.9 |
| III C9 | 330 | 84.5 | 25.6 |
| III C11 | 293 | 11.1 | 20.8 |
| III S"12 | 407 | 60.0 | 14.7 |
| III S"13 | 258 | 62.9 | 24.4 |
| III S"15 | 97 | 36.5 | 37.6 |
| IV S"1 | 335 | 68.6 | 20.5 |
| IV S"4 | 110 | 21.4 | 19.4 |
| IV S"5 | 206 | - | - |
| IV S"B7 | 174 | 60.0 | 34.5 |
| IV S"B8 | 218 | - | - |

Table 36. (Continued)

| Animal | Final weight (gm.) | Weight of gastrointestinal tract (gm.) | Weight due to gastrointestinal tract (%) |
|----------|--------------------|--|--|
| IV S"BA9 | 280 | 84.4 | 30.1 |
| IV C10 | 256 | 71.1 | 27.8 |
| IV C11 | 444 | 72.9 | 16.4 |
| IV C12 | 293 | 65.7 | 22.4 |
| IV CA13 | 371 | 61.2 | 16.5 |
| V S"B3 | 575 | 96 | 16.7 |
| V S"B4 | 572 | 86 | 15.0 |
| V S"B5 | 515 | 80 | 15.5 |
| V S"B6 | 569 | 82 | 14.1 |
| V S"B7 | 537 | 103 | 19.2 |
| V S"A8 | 390 | 104 | 26.7 |
| V S"A9 | 471 | 70 | 14.9 |
| V S"A10 | 620 | 92 | 14.8 |
| V S"A11 | 642 | 104 | 16.2 |
| V S"A12 | 584 | 95 | 16.3 |
| V S"A13 | 450 | 102 | 22.7 |
| V S"A14 | 346 | - | - |
| V C15 | 615 | 113 | 18.4 |
| V C16 | 636 | 106 | 16.7 |
| V C17 | 500 | 95 | 19.0 |
| V C20 | 636 | 105 | 16.5 |
| V C21 | 672 | 106 | 15.8 |
| V CA23 | 579 | 101 | 17.4 |
| V CA24 | 628 | 128 | 20.4 |
| V CA25 | 525 | 101 | 19.2 |
| V CA26 | 586 | 90 | 15.3 |
| V CA27 | 587 | 93 | 15.8 |
| V CA28 | 515 | 85 | 16.5 |

Table 36. (Continued)

| Animal | Final weight (gm.) | Weight of gastrointestinal tract (gm.) | Weight due to gastrointestinal tract (%) |
|----------|--------------------|--|--|
| VI S"B1 | 281 | 80.5 | 28.6 |
| VI S"B2 | 245 | 62.5 | 25.5 |
| VI S"B3 | 429 | 71.0 | 16.6 |
| VI S"B4 | 253 | 63.5 | 25.1 |
| VI S"B6 | 374 | 122.0 | 32.6 |
| VI S"B7 | 246 | 91.0 | 37.0 |
| VI S"B8 | 279 | 86.0 | 30.8 |
| VI S"B9 | 359 | 76.0 | 21.2 |
| VI S"B10 | 240 | 61.0 | 25.4 |
| VI S"B11 | 282 | 69.0 | 24.5 |
| VI S"B12 | 295 | 82.0 | 27.8 |
| VI S"B13 | 316 | 78.5 | 24.8 |
| VI S"A14 | 234 | 45.5 | 19.2 |
| VI S"A15 | 185 | 42.0 | 22.7 |
| VI S"A16 | 176 | 65.0 | 36.9 |
| VI S"A17 | 179 | 69.5 | 38.8 |
| VI S"A18 | 281 | 57.5 | 20.5 |
| VI S"A19 | 167 | - | - |
| VI S"A22 | 169 | 51.0 | 30.2 |
| VI S"A24 | 143 | 31.6 | 22.1 |
| VI S"A25 | 209 | 57.0 | 27.3 |
| VI S"A26 | 159 | 49.0 | 30.8 |