penicillin-resistant and susceptible strains of *S. aureus* were obtained. These strains were isolated before and after penicillin therapy.

The following procedure was used in testing the cross resistance of these strains:

All reagents were diluted either in casein hydrolysate or extract broth. The standard test inoculum for all strains was 200,000 bacteria per ml. The strains were checked for their resistance or susceptibility to penicillin, streptomycin or sulfonamides, respectively. All of the strains were tested against various dilutions of furacin to determine whether or not there was any difference in susceptibility to this drug of the resistant and susceptible strains. Daily visual observations of growth were made for 4 days and the results recorded as negative (no visible growth), doubtful, 1+, 2+, 3+ or 4+ growth. Casein hydrolysate medium was used for testing the penicillin and sulfonamide strains, extract broth for testing the streptomycin strains.

Results. In Table I representative results are given for each of the drugs tested. Not all of the results are given. Those omitted were essentially similar in character to those given in Table I. Except for some of the penicillin strains, a fairly high order of resistance to the various drugs tested was obtained. None of the paired organisms resistant to sulfathiazole, streptomycin or penicillin showed any change in resistance to furacin.

In most cases identical results with furacin were obtained with both the resistant and susceptible members of a pair of organisms. In some cases at the end of 48, 72, or 96 hours, differences were observed in the highest dilution inhibiting growth; that is, one member of a pair would show growth only at the next lower dilution. This reaction was just as likely to occur in the resistant as the susceptible strain of a pair of organisms.

Several experiments were tried with pairs of pneumococci[1b] resistant to sulfathiazole, acriflavine, atabrine and optochin. However, these strains grew at the highest possible concentration of furacin (1:5000), both under aerobic and anaerobic conditions, with minute inocula. The relative resistance to furacin, therefore, could not be ascertained.

Summary and Conclusions. Several Gram-negative and Gram-positive organisms resistant to sulfonamides, streptomycin and penicillin and the parent susceptible strains were tested in parallel for their susceptibility to furacin. No differences were observed between the resistant and susceptible strains in regard to their susceptibility to furacin. Resistance to penicillin, streptomycin or sulfathiazole does not result in any change to furacin resistance.

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The Corneal and Lenticular Changes Resulting from Amino Acid Deficiencies in the Rat.

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Corneal vascularization is a tissue change which may result from certain nutritional deficiencies.[1] Totter and Day[2] observed that this change resulted in the rat from deficiencies of tryptophane or lysine. Maun, Cahill and Davis found that corneal vascularization

[1] Dann, W. J., and Darby, W. J., *Physiol. Rev.* 1945, **25**, 326.

[2] Totter, J. R., and Day, P. L., *J. Nutrition*, 1942, **24**, 159.

appeared in deficiencies of leucine[3] and of histidine[4] but no histological changes in the cornea were observed when rats were made deficient in phenylalanine.[5] Methionine[6] or protein deprivation[7] may similarly produce marked corneal changes, one of the most obvious of which is corneal vascularization. The suggestion has been made that corneal vascularization may be a reaction resulting from a deficiency of any of the indispensable amino acids or of protein.[8] In the following report is an account of the corneal changes which we have observed to occur in deficiencies of certain of the indispensable amino acids.

Methods. Rats from a Wistar strain which were 25 or 26 days of age were placed in individual cages and given an experimental diet and water *ad libitum*. The control diets contained an amino acid mixture as used by Maun, Cahill and Davis[5] in their control diet. This supplied approximately 3 times the amount required for normal growth of each of the indispensable amino acids. The remainder of our control diet contained in each 100 g: salt mixture[9] 4 g, cod liver oil 2 g, cottonseed oil 3 g, choline chloride 0.2 g, riboflavin 1.6 mg, thiamin hydrochloride 0.4 mg, calcium pantothenate 2 mg, pyridoxin hydrochloride 0.4 mg and sufficient sucrose to make 100 g. Diets deficient in phenylalanine, leucine, isoleucine, arginine, histidine, threonine and valine were prepared by replacing the appropriate amino acid in the formula of the control diet with sucrose. Eight litters of 6 rats, one litter of 4, and one of 5 rats were distributed among the diets so that there were 7 rats from different litters on each diet except for 8 on the threonine-deficient diet. Biomicroscopic examination of the rats' eyes were made 3 or 4 times weekly. One rat from each group where corneal vascularization occurred was changed to the control diet to induce regression of the vascularization. Further details of technics used here were described in an earlier paper.[10] As before, we limit our use of the term corneal vascularization to the situation where there is an actual growth of capillaries in the cornea beyond the limits of normal variation.

Results. In all of the amino acid deficiencies investigated in this study some degree of corneal vascularization occurred. It was the most prompt and extreme in phenylalanine deficiency although the vascularization resulting from isoleucine and leucine deficiencies was nearly as extreme. Successively lesser degrees of corneal vascularization were observed in histidine, valine, threonine and least in arginine deficiencies. Of the 7 animals in each group on deficient diets the number which have showed corneal vascularization are respectively: phenylalanine 7, leucine 6, isoleucine 7, arginine 5, histidine 7, threonine 7 (of 8 rats), and valine 5. One of the control rats developed a few capillary loops into the edge of the cornea, beyond the usual limits of variation. Rats deficient in leucine, isoleucine, phenylalanine, histidine and threonine, when returned to the control diet to supply the lacking amino acid, underwent a prompt regression of vascularization until no corneal capillaries could be seen with the biomicroscope.

The observations described above as to the corneal vascularization occurring in leucine and histidine deficiencies are in accord with the findings of Maun, Cahill and Davis[1,2] in these deficiencies. Our findings in phenylalanine deficiency are in contradiction to those of Maun, Cahill and Davis,[3] since they reported that "neither the eyes

[3] Maun, M. E., Cahill, W. M., and Davis, R. M., *Arch. Path.*, 1945, **40**, 173.

[4] Maun, M. E., Cahill, W. M., and Davis, R. M., *Arch. Path.*, 1946, **41**, 25.

[5] Maun, M. E., Cahill, W. M., and Davis, R. M., *Arch. Path.*, 1945, **39**, 294.

[6] Berg, J. L., Pund, E. R., Sydenstricker, V. P., Hall, W. K., Bowles, L. L., and Hock, C. W., *J. Nutrition*, in press.

[7] Hall, W. K., Sydenstricker, V. P., Hock, C. W., and Bowles, L. L., *J. Nutrition*, 1946, **32**, 509.

[8] Sydenstricker, V. P., Hall, W. K., Hock, C. W., and Pund, E. R., *Science*, 1946, **103**, 194.

[9] McKibben, J. M., Madden, R. J., Black, S., and Elvehjem, C. A., *Am. J. Physiol.*, 1939, **128**, 102.

[10] Bowles, L. L., Allen, L., Sydenstricker, V. P., Hock, C. W., and Hall, W. K., *J. Nutrition*, 1946, **32**, 19.

of the deficient, nor those of the control animals showed alterations."

From the work reported or cited here it appears that corneal vascularization is a reaction which results in the rat from a deficiency of any of the indispensable amino acids or from protein deprivation.

To date, 4 of the 7 rats on the histidine-deficient diet have developed bilateral cataracts of varying size. It is to be noted that these 4 rats are all from different litters and that none of the rats on the other diets used in this study have shown any lenticular changes which could be seen with the biomicroscope.

Summary. In the rat, deficiencies of leucine, isoleucine, phenylalanine, histidine, threonine, valine, or of arginine, resulted in corneal vascularization. Four of 7 rats on a histidine-deficient diet developed lenticular opacities.

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Chemical Changes in the Developing Turtle Embryo.

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It has been found in previous experiments[1] that the embryo of the snapping turtle (*Chelydra serpentina*) consumes fat in the amount of 61 mg per individual during the last 57 days of its development. Data obtained on the loss of dry substance during the same period, in conjunction with figures for fat loss and oxygen consumption, led to the assumption that relatively large amounts of protein were probably metabolized also. In the present study determinations of protein, carbohydrate, inorganic substances, dry substance and fat were made on turtle eggs at the beginning of development and on newly hatched turtles. The eggs used were collected from the oviducts of 3 large snapping turtles on June 11, 1946 and each egg was weighed individually. Fifteen of the eggs (5 from each clutch) were opened at once and the egg contents (albumen and yolk) of all 15 were put into a single vessel and dried at 110°C to serve for the determination of the initial chemical composition. The other eggs were incubated at room temperature and when the young turtles hatched (72-77 days) each was weighed and 15 specimens (5 from each clutch, as in the initial egg batch) were placed in a vessel and dried at 110°C. Eggs of as nearly as possible the same initial weights were used. The uniformity of the material is indicated by the fact that the average weight of the 15 eggs used for analysis of the chemical composition at the beginning of development was 11.13 ± 0.11 g (eggshells included) while the initial average weight of the 15 eggs which gave rise to the hatched turtles analyzed was 11.10 ± 0.09 g.

The 2 lots of dried material were each ground to a fine powder in a grinding mill and the following fractions were determined in aliquot parts by the methods specified: nitrogen according to Kjeldhal, using the customary factor of 6.25 to convert to protein; fat according to Kumagava-Suto;[2] total carbohydrate according to Dische and Popper[3] and inorganic substances by inciner-

[1] Lynn, W. G., and von Brand, T., *Biol. Bull.*, 1945, **88**, 112.

[2] Kumagava, M., and Suto, K., *Bioch. Z.*, 1908, **8**, 212.

[3] Dische, Z., and Popper, H., *Bioch. Z.*, 1926, **175**, 371.