of the eyes of three rabbits. Two of them developed marked iritis and also a systemic reaction indicated by diarrhea of several days' duration; the third had a panophthalmitis with some form of cocci as contaminating agents. Another set of three rabbits was inoculated with the seventh and eighth subcultures from an arthritic exudate (this culture had never undergone animal passage); one rabbit developed marked iritis and diarrhea, the second mild iritis; while the eye of the third has so far remained free from macroscopic lesions. Two out of three other rabbits inoculated with a 24-hour-old culture of the same strain developed definite iritis after 9 or 10 days; while the iritis in the first two groups appeared between the second and fifth days after inoculation and persisted from the seventh to tenth.

Four series of Swiss mice, of a stock known to be free from mouse typhoid infection, were inoculated intranasally with the same cultures that had been injected into rabbits' eyes. During the following six days, animals in each set were obviously sick and had dyspnea. When autopsied on the sixth or seventh days, 3 out of 5 mice inoculated with the 4-day-old culture showed only macroscopically equivocal pulmonary lesions. On the other hand, marked pneumonia was present in 2 out of 5 mice in each of the three sets inoculated with either 1- or 2-day-old cultures. Another macroscopically normal appearing mouse lung was found upon microscopic examination to have foci of interstitial pneumonia, perivascular hyperplasia and bronchi distended with polymorphonuclear cells, a picture that has been peculiar to all the pneumonic lungs examined.

It thus appears that pleuropneumonia-like microorganisms cultured directly from rheumatic exudates can induce the same type of pneumonia in mice that is obtained by inoculating these animals with rheumatic exudates, or with suspensions of chorioallantoic membranes in which characteristic lesions have been induced by these exudates. These pulmonic lesions have appeared in the first mice inoculated with these various materials, as well as in those where serial transfers have been carried out; hence we feel that the organotropism of these microorganisms is different from those of the pleuropneumonia-like microorganisms recovered from mice by Dr. Sabin, for he has been unable to induce pneumonia in mice with his cultures.[7] A few mice inoculated either intracerebrally, intravenously or intraperitoneally with cultures have, so far, shown no characteristic lesions, even though some of them have been obviously sick. The series has been too small, however, and the time since inoculation too short for final judgment concerning the pathogenicity of these cultures.

SUMMARY

In suitable cell-free media it has been possible to cultivate pleuropneumonia-like microorganisms from the following materials, first, from chorioallantoic membranes in which lesions were apparently induced by exudates from patients with rheumatic fever; second, from pneumonic lungs of mice inoculated with similar exudates or with suspensions of the above-mentioned abnormal membranes; and third, directly from the arthritic exudate of a patient with rheumatic fever, and also from an erythema nodosum nodule excised from a patient with this same disease. With three different subcultures from joint fluid, iritis has been induced in rabbits; and following intranasal inoculation with the same cultures there has developed in mice a pneumonia similar to that found in mice inoculated with rheumatic exudates and with suspensions of chorioallantoic membranes infected with rheumatic exudates. Therefore it seems probable that in all instances the pathogenic agent was derived from similar sources, *viz.*, patients with rheumatic fever. Further work will be required to demonstrate the etiologic significance of these pathogenic agents in rheumatic fever.

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THE PROCESS OF CONTINUOUS DEAMINATION AND REAMINATION OF AMINO ACIDS IN THE PROTEINS OF NORMAL ANIMALS

WE have shown[1] that the feeding of *dl*-tyrosine with an increased concentration of the nitrogen isotope N^{15} to normal rats kept in nitrogen equilibrium leads not only to the incorporation of isotopic tyrosine into the tissue proteins, but also to the formation of other isotopic amino acids. This transfer of the nitrogen from one protein constituent to others could only have been due to chemical reactions, one of which must have involved the opening of peptide linkages. It was suspected that the mechanism responsible was that of deamination of tyrosine (to the corresponding α-keto acid?) coupled with the amination of another substance (α-keto acid?) to form the new amino acid. A process of this type was first proposed by Braunstein and Kritzman[2] and demonstrated in minced muscle. In our experiments the transfer of N^{15} from tyrosine into the α-amino group, but not into the ring,[3] of histidine offered strong evidence for the hypothesis that the shift of nitrogen from one amino acid to another is a normal event, which occurs at a rapid rate.

However, the possibility was not excluded that the

[7] A. B. Sabin, personal communication.

[1] R. Schoenheimer, S. Ratner and D. Rittenberg, *Jour. Biol. Chem.*, 127: 333, 1939.

[2] A. F. Braunstein and M. G. Kritzman, *Enzymologia*, 2: 129, 1937.

[3] R. Schoenheimer, D. Rittenberg and A. S. Keston, *Jour. Biol. Chem.*, 127: 385, 1939.

transfer of the nitrogen of one amino acid into other amino acids is also associated with a more fundamental synthesis of the carbon chain of the latter. We are now able to offer proof of the occurrence of extensive deamination and reamination of amino acids of the proteins in normal animals, by following the fate of the amino acid *l*-leucine, which contained two different and independent isotopes, namely, deuterium in the carbon chain and N^{15} in the amino group.

Four adult rats were kept on an ordinary stock diet (containing 15 per cent. casein) in nitrogen equilibrium without change of weight. To the diet was added an amount of *l-leucine* corresponding to 23 mg of nitrogen per rat per day for three days; the animals were then immediately killed by exsanguination. The leucine (possessing the natural configuration) contained 3.6 atom per cent. deuterium and 6.5 atom per cent. N^{15}; it was obtained by resolution of the synthetic racemic mixture described before.[4] While the total amount of nitrogen excreted corresponded to that in the diet, the excreta contained only 30 per cent. of the administered isotopic nitrogen, most of the remainder being incorporated in the body proteins. Only 8 per cent. was found in the "non-protein nitrogen" of the tissues.

The proteins of the liver, of the intestinal wall and of the remaining carcass were worked up separately, and the following analytically pure amino acids were isolated from the different proteins: three preparations each of arginine, tyrosine, glutamic acid, aspartic acid and leucine; two preparations of glycine and lysine, and one preparation of ornithine obtained by degradation of liver arginine. All the compounds, with the exception of the two lysine preparations, contained appreciable amounts of nitrogen isotope, a finding which corroborates our earlier observations after feeding tyrosine. The isotope concentration in the three leucine preparations was considerably higher than that of any other amino acid obtained from the same protein.

Indication of the mechanism responsible for the nitrogen transfer was obtained by the closer investigation of the leucine isolated from the proteins of the animals. The isotope (D and N^{15}) concentrations of the leucine isolated were considerably lower than those of the material fed. This was to be expected, as the dietary leucine was mixed with the ordinary leucine of the casein and with that of the tissue proteins into which the dietary leucine was introduced. If the leucine isolated from the tissues were only "diluted" by this "ordinary leucine," the ratio of the concentrations of the two isotopes in the compound should have been the same as in the material fed. The ratio (D : N^{15}), however, was altered considerably. It was 100 : 167 in the leucine administered and 100 : 103 in that isolated from the carcass. This result indicates that the carbon chain of leucine (characterized by the labeled hydrogen) had given up part of its nitrogen (in the present case labeled nitrogen) and in turn had "accepted" new nitrogen (which was normal). The other amino acids were obviously also involved in this process, in that they gave up normal nitrogen and accepted isotopic nitrogen from leucine.

The rapid introduction of a dietary amino acid into the tissue proteins, as well as the rapid and continuous deamination and amination of a large number of amino acids demonstrated in proteins of different organs has here taken place in normal adult animals on a stock diet. As all these reactions require the opening of peptide linkages in the protein, the finding is a new indication of the high chemical activity of the tissue proteins. The experimental details and their physiological and chemical implications will be presented elsewhere.

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[4] R. Schoenheimer and S. Ratner, *Jour. Biol. Chem.*, 127: 301, 1939.

A METHOD FOR PRODUCING PERSISTENT HYPERTENSION BY CELLOPHANE

IT has been found that arterial hypertension can be produced in dogs by wrapping one or both kidneys in Cellophane.

Dogs were anesthetized, a kidney freed from its bed, and after stripping off the fat on the surface, Cellophane sterilized in alcohol was gently wrapped around it and secured either with paper clips or a ligature. It is not necessary that very accurate approximation of the kidney and Cellophane occur. The kidney is gently replaced and the wound closed. This procedure may be repeated on the opposite kidney.

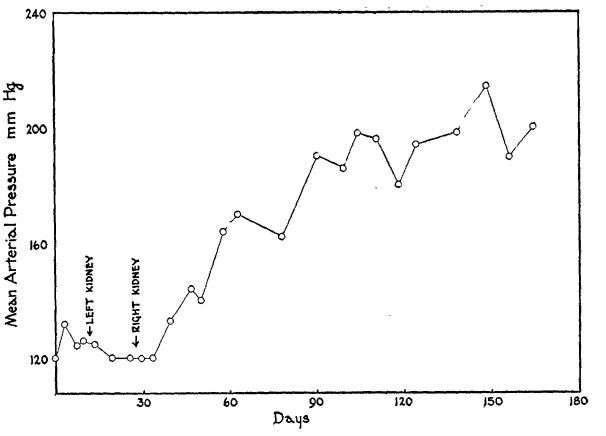

FIG. 1. Both kidneys were placed in Cellophane and blood pressure measured by intra-femoral puncture.