duced decided vaso-motor reactions in other parts of the body a few minutes previous to these experiments, they remained ineffective when introduced into the pulmonary circuit.

41 (184)

The effect of salicylic acid upon autolysis.

By **L. B. STOOKEY.**

[*From the Physiological Laboratory, Medical Department, University of Southern California.*]

The liver, kidney, spleen and muscle taken from dogs which had received subcutaneously doses of sodium salicylate (0.1 gram, in 1 per cent. solution, per kilo of body weight) daily, during a period of ten days, showed rates of autolysis greater than those observed in the same organs taken from normal dogs.

The influence of other drugs upon autolysis is being investigated.

42 (185)

On the synthesis of protein through the action of trypsin.

By **ALONZO ENGLEBERT TAYLOR.**

[*From the Laboratory of Pathology, University of California.*]

The application of the theory of thermodynamics to general chemical reactions has resulted in the definition of the following principles, all of which have been confirmed by experiment as well as by mathematical considerations:

All chemical reactions are reversible reactions;

All chemical reactions progress to an equilibrium in the system.

There is in every chemical reaction a driving force and an internal chemical resistance.

Catalytic acceleration operates through a reduction in the internal chemical resistance; since the driving force is unaltered, the station of equilibrium is attained more quickly, that is, the experimental velocity of the reaction is increased.

The catalytic acceleration operates in either direction of the reaction; no matter in which direction the reaction may happen

to be proceeding at a particular moment, the catalyser accelerates the progress to the station of equilibrium.

On the basis of these considerations van't Hoff ten years ago predicted that the common reactions of fermentation were reversible if the appropriate conditions could be secured. This would mean the synthesis of organic substances through the acceleration of the reversed reactions, and he expressed the suggestion that the syntheses in nature might be regarded as such.

Of the three large groups of organic substances conspicuous in the living plant or animal body, *i. e.*, carbohydrates, fats and proteins, successful reversions have been accomplished in but the first two. Of carbohydrates the following have been synthesized by ferment action: starch, glycogen, cane sugar, maltose, lactose and glucosides. Fats of both the mon-atomic alcohols and of glycerol have been synthesized by ferment action. Two years ago I published the details of a long series of failures at the synthesis of protein. Since that time I have attempted repeatedly to effect the synthesis of the peptids of Fischer through the action of trypsin. The results were entirely negative. Recently Abderhalden has published the negative results of a similiar set of experiments. Not long ago I succeeded in an effort to effect the synthesis of a protein through the action of trypsin. The detailed description of the work, of which this is but a preliminary announcement, will be published in the *Journal of Biological Chemistry.*

Four hundred grams of the protamin sulphate of the striped bass were digested with trypsin until the hydrolysis of the substrate was completed. At the close of the digestion, the solution was miscible with five volumes of acidulated alcohol without the production of any opacity, and gave with cold saturation with sodium chloride no precipitation. This solution was then heated to the boiling point, freed of its sulphuric acid by the addition of barium hydroxid, the excess of barium removed by saturation with carbon dioxide, the mixture filtered hot and filtration repeated until the fluid was clear. This solution then represented a solution of the amino acids, free and combined with carbon dioxide, the products of the hydrolysis of the protamin. The solution was clear, and had an alkaline reaction. This solution was then concentrated until the beginning of precipitation in the cold, from which it was inferred

that the solvent was saturated with the products of the digestion, a theoretically favorable condition for the reversed reaction. To this was then added 300 c.c. of a glycerol extract of livers from large, soft shelled, California clams, which contain a strong tryptic ferment. The solution was then miscible with alcohol without cloudiness. Twenty c.c. of toluol were then added, and the flask, containing over four litres, then sealed and set aside. As time passed this solution became opalescent, then cloudy, and finally a fine white precipitate settled on the bottom of the flask. Five months after the experiment was begun the flask was opened, heated to the boiling point to destroy the ferment, acidulated with sulphuric acid, which dissolved the white precipitate, filtered and then precipitated by the addition of four volumes of absolute alcohol. A heavy, white precipitate was produced, which was collected by filtration, washed with alcohol, redissolved in water, reprecipitated by alcohol, and this procedure repeated four times. The final white powder when fully purified and dried weighed 1.8 gram. Probably one fourth of the amount had been lost in the processes of purification. This powder was soluble in water up to a concentration of about three per cent., was precipitated by acidulated alcohol, and was salted out of a ten per cent. solution of sodium chloride. It was analyzed for carbon, hydrogen, nitrogen, and sulphuric acid. The results of these analyses agree well with the known composition of the protamin sulphate. This for the protamin of the striped bass I long ago determined to be $C_{30}H_{60}N_{17}O_6 \cdot 2H_2SO_4$. Calculated according to this formula, the theoretical percentages and the percentages determined in the analyses were as follows:

	Calculated.	Found.
C	37.85%	37.68%
H	6.72%	6.89%
N	25.13%	24.45%, 24.93%, 25.06%, 25.18%
H_2SO_4	20.60%	20.68%

The conclusion is obvious that the substance formed was protamin.

I had previously carried out experiments with protamin, but always with negative results. The positive result in this experiment must have been due to one of two circumstances. Either to the use of this particular ferment, which is very resistant, or to the use of

the free amino acids and the carbonates, instead of the sulphates, as previously. Future experiments must determine which. A control, a fraction of the original solution without the ferment, has not changed during the time of the experiment. The glycerol extract used, some of which was preserved, is still active; the ferment is therefore very long lived. A culture made of the experimental material at the close of the experiment was negative.

43 (186)

A method for separating leucin from amino-valerianic acid.

By **P. A. LEVENE.**

[*From the Rockefeller Institute for Medical Research.*]

Separation of leucin from amino-valerianic acid was accomplished by means of lead acetate and ammonia. A basic lead salt of leucin, insoluble in hot water, was formed. From a mixture containing 52.53 per cent. of C and 9.39 per cent. of H, by the use of these reagents, a substance was obtained, which had 54.55 per cent. of C and 9.90 per cent. of H. On reprecipitation it acquired the composition: C = 54.70 per cent.; H = 10.09 per cent. Leucin contains 54.89 per cent. of C and 10.01 per cent. of H.