or in a new combination VIII . . .

An O-antiserum prepared with boiled culture material agglutinated the homologous strain and *SS. newport, kentucky, amherstiana* almost uniformly to its end titer, but did not attack types with the VI.VII, VI.XIV.XXIV, and I.VI.XIV.XXV antigens. VIII containing types (*SS. newport, kentucky, amherstiana*) removed all antibodies for themselves and for the new strain from the serum. Thus, the exclusive presence of the VIII antigen and its identity with that of the other types could be established. The newly found VIII seems even more complete than that of *S. amherstiana* which in Edwards and Bruner's experience did not fully cover the VIII of the *newport* type.

The H-antigen. The strain's flagellar antigen is a monophasic *d* as is present in *S. typhi* and *S. wichita*. Testing of numerous single colonies did not reveal another phase, nor did the culturing in semi-solid agar with addition of *d* antiserum (Gard's technic). Living and formolized antigens are readily agglutinated by sera prepared with types endowed with the *d* antigen (*SS. stanley, amersfoort, muenchen, typhi, shangani, niloese, wichita*). The sera agglutinate the strain 2255 to approximately 50% of their relative titers. On the other hand, this strain removes about 75% of their agglutinins by absorption. A serum prepared with formolized broth culture of the strain agglutinates all *d* containing types to high titers, some of them to the end titer. Slight differences observed are in agreement with previous statements by Kauffmann,[2] Hormaeche and Peluffo,[3] and Edwards and Bruner.[4] These authors found differences in the *d* of different types and emphasized the complexity of that flagellar antigen.

The strain 2255 represents a new pathogenic Salmonella type. It has been named *S. virginia* after its place of isolation.

Summary. *S. virginia,* a new Salmonella type, has been described. It was isolated from a case of human enteritis and has the formula VIII; d —.

[2] Kauffmann, F., *Z. Hyg.*, 1937, **120**, 177.

[3] Hormaeche, E., and Peluffo, C. A., *Arch. Urug. de Med. Cir y Esp.*, 1941, **14**, 217.

[4] Edwards, P. R., and Bruner, D. W., *Am. J. Hyg.*, 1942, **34**, 121.

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Cystine Inhibition of the Succinoxidase System. III. Effect of Dialysis.*

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During the course of a previous investigation on the inhibition of the succinoxidase system by cysteine and cystine, we showed that the concentration of four-carbon dicarboxylic (C-4) acids in the tissue homogenate was a determining factor.[1] Subsequently, we showed that with either cysteine or cystine added to the reaction mixture (cytochrome *c* present), the inhibition was entirely due to cystine.[2]

All inhibitors acting on the sulfhydryl group in succinic dehydrogenase will be affected by the presence of any C-4 acids or related substances. Since considerable importance has been attributed to the action of such inhibitors in determining whether or not enzymes function by means of sulfhydryl groups,[3,4] some means must be found to eliminate these interfering substances from the

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[1] Ames, S. R., and Elvehjem, C. A., *Arch. Biochem.*, 1944, **5**, 191.

[2] Ames, S. R., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 109.

[3] Potter, V. R., and DuBois, K. P., *J. Gen. Physiol.*, 1943, **26**, 391.

[4] Barron, E. S. G., and Singer, T. P., *Science*, 1943, **97**, 356.

homogenate before quantitatively accurate data can be obtained.

It is the purpose of this communication to discuss an attempt to remove such interfering substances by means of dialysis. While much extraneous material and a considerable portion of the C-4 acids present were removed, an inhibition approaching 100% could not be secured under the experimental conditions.

White mice of an inbred Swiss strain were used and, after weaning, were maintained on stock ration† and water *ad libitum* plus occasional greens. Adult male white rats of the Sprague-Dawley strain were also used and were maintained on the same ration as the mice.

Tissue homogenates were prepared by the technic of Potter and Elvehjem[5] modified as described previously.[1,2] The activity of succinic dehydrogenase was determined by the method of Potter[6] as modified by Potter and Schneider.[7]

In order to secure reproducible results the *incubation time, i.e.*, the time the homogenate was incubated at 37°C before the inhibitor was added, and the *contact time, i.e.*, the time the inhibitor was in contact with the enzyme before the substrate was added, were carefully controlled. The numerical inhibitions recorded are valid only for a similar preparation under identical conditions.

A method of dialysis was selected in which the sample was constantly in motion.‡ By this means dialysis was facilitated, thus shortening the time of dialysis and subsequent losses in activity. Regenerated cellulose tubing§ was used as the dialysis membrane. Redistilled water was used and was frequently changed. The temperatures were roughly maintained as indicated by means of an external water or ice bath. The dry weights of the samples were determined by evaporating to constant weight in open crucibles in an electric oven at 110°C.

The cystine was recrystallized by the method previously described.[2] A stock solution of 0.72 mg per ml in 0.05 M sodium phosphate buffer was prepared by heating to boiling and this solution remained supersaturated for 12 hours or more at room temperature.

The temperature at which the homogenate was dialyzed proved to be an important factor in determining activity losses. A comparison of Curves I and II of Fig. 1 shows that the lower the temperature the less the loss in activity. At 25°C no activity remained after 6 hours of dialysis of a 2% homogenate, whereas at 0°C a similar preparation exhibited 64% of its original activity after 6 hours dialysis. Similar results were obtained on storage of homogenates *in vitro*. Twelve

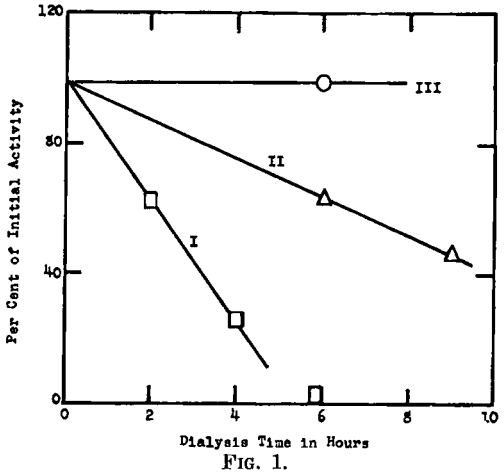

FIG. 1.

Effect of dialysis on the total succinoxidase activity. Assay performed as described in text. Incubation time of 40 minutes and contact time of 30 minutes with no inhibitor added.

Curve I—2% homogenate of mouse kidney dialyzed at 25°C.

Curve II—2% homogenate of mouse kidney dialyzed at 0°C.

Curve III—10% homogenate of mouse kidney dialyzed at 0°C.

† B-B Laboratory Rabbit Diet, Maritime Milling Company, Inc., Buffalo, New York.

5 Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, **114**, 495.

6 Potter, V. R., *J. Biol. Chem.*, 1941, **141**, 775.

7 Potter, V. R., and Schneider, W. C., *J. Biol. Chem.*, 1942, **142**, 543.

‡ A weighted dialysis bag containing the sample was suspended in water from a rubber band which was stretched from a fixed support to an eccentric rod rotating at about 100 r.p.m. This stirring rod was so bent that the rubber band was stretched about 4 cm with each revolution. By this means the suspended dialysis bag was alternately raised and lowered, this resulting in a "milking" effect which compensated in part for the normal increase in volume of the dialysate.

§ Visking cellulose sausage casings, The Visking Corporation, Chicago, Illinois.

TABLE I.
Dialysis and Storage of Tissue Homogenates.

Exp.	Description	% inhibition		% increase in inhibition	Time in hrs	% increase in inhibition per hr
		Before treatment	After treatment			
1	Mouse kidney—2% homogenate dialyzed at 25°C	41	56	37	4	9.2
2	Mouse kidney—2% homogenate dialyzed at 0°C	42	57	36	6	6.0
3	Mouse kidney—10% homogenate dialyzed at 0°C	42	55	31	7½	4.1
4	Mouse kidney—10% homogenate stored at 0°C	42	45	7	7½	0.9
5	Mouse kidney—10% homogenate stored at 0°C with the addition of cytochrome *c* to 10^{-4} M and coenzyme I to 75 γ/ml	40	53	32	3½	9.3
6	Rat liver—10% homogenate dialyzed at 0°C	19	28	47	5½	8.6
7	Rat liver—10% homogenate stored at 0°C	19	28	47	5½	8.6

Assay performed as described in text. Incubation time of 40 minutes and contact time of 30 minutes with a cystine concentration of M/2000.

hours storage of a 10% homogenate of rat liver at 8°C resulted in complete loss of activity, but on freezing a similar preparation and maintaining it at −5°C for 12 hours, no losses were observed. It is quite evident, therefore, that the lower the temperature, the more stable is the enzyme and even a few degrees above zero is sufficient for total inactivation.

The dilution of the homogenate to be dialyzed was another factor which had to be taken into account. Curves II and III of Fig. 1 illustrate the changes in total activity resulting from the dialysis of a 2% and a 10% homogenate respectively at 0°C. The 10% homogenate showed no loss in activity while the 2% homogenate was about 50% inactivated in 7 hours, indicating that if the total concentration of protein was reduced, the inactivation increased. These results are similar to those obtained by Nelson and coworkers[8,9] for purified enzymes. These workers showed that the spontaneous inactivation which occurred in dilute solutions of tyrosinase and ascorbic acid oxidase could be reduced by adding solutions of an inert protein, such as gelatin. The above experiments indicate that the dialysis of a 10% homogenate at 0°C would result in little or no loss of succinoxidase activity during the course of 6 hours.

In order to compare the rates at which the C-4 acids were removed under varying conditions and times of dialysis, a method for expressing the observed data was selected in which a rate of change in the inhibition was computed. This value furnished a ready means of comparing results since it was proportional to the rate of disappearance of C-4 acids per unit time. The results included in Table I were calculated to give the average percent increase in the percent inhibition per hour over the time interval indicated, based on the initially obtained percent inhibition. Such a comparison is valid since the rate is approximately a linear function of the time.

We were concerned in these experiments (Table I) primarily with the speed of disappearance of the factors which influenced the degree of inhibition. Exp. 1 and 2 show clearly how the change in inhibition varied with temperature. At the higher temperature dialysis was more rapid as shown by the higher numerical change in the inhibition. Similarly, Exp. 2 and 3 illustrate the changes observed with a 2% and a 10% homogenate respectively and demonstrate that in the more concentrated homogenates, the substances affecting the inhibition were removed more slowly.

[8] Adams, M. H., and Nelson, J. M., *J. Am. Chem. Soc.*, 1938, **60**, 2472.

[9] Lovett-Janison, P. L., and Nelson, J. M., *J. Am. Chem. Soc.*, 1940, **62**, 1409.

Since all previous experimental work had been performed on homogenates of mouse kidney, rat liver homogenates were subjected to similar treatment for comparative purposes. Contrary to previous preliminary observations,[1] these two tissues seemed to differ widely, both in the degree of inhibition and in the rate of disappearance of the factors affecting the inhibition. The rate for rat liver homogenates was almost double that of a homogenate of mouse kidney, and in addition, the extent of inhibition was just about half, 42% and 19% inhibition respectively.

It was not evident whether the results observed were due solely to dialysis or whether the series of reactions already known to function in the homogenate[1] was a contributing factor. Parallel experiments were performed in which a portion of the homogenate was placed in a tube and maintained under the same conditions as the portion dialyzed. In the case of mouse kidney, a comparison of Exp. 3 and 4 of Table I shows that on storage the removal of the substances interfering with the normal inhibition of the enzyme occurs slowly but by no means as rapidly as on dialysis of the homogenate. On the contrary, in rat liver preparations dialysis did not increase the removal of such substances over storage alone (Exp. 6 and 7 of Table I).

An increase in inhibition on storage had been previously attributed to a destruction of the C-4 acids through the cozymase-linked malic dehydrogenase.[1] To test this hypothesis further and to see if the postulated reactions were functioning in the cold, a sample of mouse kidney homogenate was stored at 0°C and cytochrome *c* and coenzyme I‖ were added (Exp. 5 of Table I). The resultant rate of increase in inhibition with time was doubled over that obtained without addition of these two substances and was numerically similar to that obtained with rat liver preparations. A possible explanation of the differences secured with these two tissues is that mouse kidney has a lower concentration of coenzyme I and cytochrome *c* than rat liver. Thus on homogenization these substances are reduced to ineffectual concentrations in the case of the mouse kidney but still remain active in the rat liver homogenates.

A major factor in limiting dialysis as a rapid method of securing homogenates free of substances which interfere in the determination of the inhibition is that the inhibition varies inversely as the log of the C-4 acid concentration.[1] Therefore a large decrease in the C-4 acid concentration results in a small increase in the inhibition. Many of the data presented indicate a loss of 80% to 90% of the original concentration of C-4 acids in the course of about 6 hours dialysis. Dialysis can thus be used as a method of reducing the concentration of substances such as the C-4 acids which interfere with the inhibition reaction.

Summary. 1. The inhibition of the succinoxidase system by cystine can be increased by dialysis of the tissue homogenate. 2. Temperatures above 0°C and dilution of the tissue homogenates (below 10%) favor the spontaneous inactivation of this system.

‖ We are indebted to Merck and Company, Inc., Rahway, N.J., for a generous gift of cozymase.