The fibrinolytic factor formed or secreted by the Staphylococcus is not of the same immunologic specificity as the streptofibrinolysin. Streptococcus antiserum‡ containing as many as 1000 antifibrinolytic units per cc. does not neutralize the staphylofibrinolysin.

8312 C

Survival of Two Depancreatized Dogs Treated with Insulin.*

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Soon after the discovery of insulin, attempts were made to maintain completely depancreatized dogs with insulin. Macleod[1, 2, 3] found that dogs receiving insulin and a diet of meat and sucrose survived for 8 months, whereas the addition of raw pancreas to their diets permitted the survival of 2 completely depancreatized dogs for about 4 years. Although the diets employed were deficient, Macleod concluded that raw pancreas was essential for the survival of the depancreatized dogs for periods longer than 8 months. More recently Hershey[4] reported that lecithin was of value in this connection. Hershey and Soskin[5] state that the ingestion of lecithin supplements enables depancreatized dogs to live indefinitely and that it cures the hepatic insufficiency that appears from 6 weeks to 11 months after pancreatectomy. However, the longest period of survival reported by these workers was 1 year and 3 months. Later, Best and Hershey[6] and Best, Ferguson and Hershey[7] found that the

‡ The antistreptococcus serums used in these and other tests were kindly furnished by: Eli Lilly and Co.; The Cutter Laboratory; Parke, Davis and Co.; Lederle Laboratories; and E. R. Squibb and Sons.

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1 Allen, F. N., Bowie, D. J., Macleod, J. J. R., and Robinson, W. L., *Brit. J. Exp. Path.*, 1924, **5**, 75.

2 Macleod, J. J. R., Carbohydrate metabolism and insulin. Longmans, Green and Co., Ltd., New York, 1926.

3 Macleod, J. J. R., *Lancet*, 1930, **219**, 383.

4 Hershey, J. M., *Am. J. Physiol.*, 1930, **93**, 657.

5 Hershey, J. M., and Soskin, S., *Am. J. Physiol.*, 1931, **98**, 74.

6 Best, C. H., and Hershey, J. M., *J. Physiol.*, 1932, **75**, 49.

7 Best, C. H., Ferguson, G. C., and Hershey, J. M., *J. Physiol.*, 1933, **79**, 94.

essential factor in lecithin was choline. It should be noted that the longest period of survival of a depancreatized dog reported in the latter publications was somewhat more than 2.5 years.

The present report deals *only* with the period of survival of the depancreatized dog. Following complete pancreatectomy, 2 dogs were injected twice daily with insulin and fed a diet containing the nutritional requirements known to be essential for the normal dog. They received twice daily a mixture consisting of lean meat, sucrose and bone ash in amounts previously specified.[8] In addition, vitamin supplements were added twice a week, A and D in the form of cod liver oil, B as a concentrate obtained from rice polishings. On such treatment 2 depancreatized dogs have survived for well over 4 years and at present are alive. A description of these dogs follows:

Dog DA—female; completely depancreatized March 11, 1931.

Dog DC—female; born November 20, 1930; completely depancreatized September 1, 1931.

Both dogs are at present (October, 1935) in good nutritional condition. The cataractous formations in the lenses† of these dogs, as well as the state of the blood lipids,[8] have been noted elsewhere. The postabsorptive blood sugars of these animals have always been above normal, hypoglycemia having never been observed in either of these dogs during their entire stay in the laboratory.

The relation of choline to the survival of dogs DA and DC remains to be considered. It is known that the largest part of the choline present in lean meat is contained in lecithin,[9] and that the choline present in other forms is a negligible amount.[10] Both meat and cod liver oil‡ have been used by Best *et al.*[5, 7] in the diets that produced fatty livers in depancreatized dogs. It is the prevention of fatty livers which Best *et al.* claim is effected by feeding choline and which they regard as essential for the survival of such dogs for long periods. The vitamin B concentrate§ employed by us is obtained from an aqueous extract of rice polishings and is fed in such small amounts that, regardless of the amount present, in all probability it can furnish only negligible amounts of choline, far less

[8] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, **106**, 267.

† The cataracts found in dogs DA and DC have been described in the PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 237, where these 2 animals are recorded as dogs 7 and 2 respectively.

[9] Bloor, W. R., *J. Biol. Chem.*, 1927, **72**, 327.

[10] Alles, G. A., *Physiol. Rev.*, 1934, **14**, 276.

‡ According to phospholipid analysis of the cod liver oil used in this study, dogs DA and DC received negligible amounts of choline by this means.

§ 100 gm. of this extract contained less than 15 mg. of phospholipid.

than that already contained in the meat. If, as Best *et al.* claim, choline reduces the fat content of the livers of depancreatized dogs to normal levels, then the fact that the diet used in the present investigation produced livers containing as high as 43% fatty acids,[11] a value higher than that hitherto reported, is further evidence that choline could have played no part in the survival of dogs DA and DC for 4.5 and 4 years respectively.

The fact that in the present study one dog was kept alive for 4.5 years and another for 4 years lends doubtful support to the view that raw pancreas, lecithin supplements or choline supplements are essential for *maintenance of the depancreatized dog.* Indeed, dogs DA and DC, which have never received raw pancreas, lecithin supplements or choline supplements during their entire stay in this laboratory, have lived longer than depancreatized dogs reported to have received either one or other of these 3 constituents. It is obvious, therefore, that a minimum period of 4.5 years must elapse in the survival of a depancreatized dog before it can be claimed that a given substance is essential in keeping it alive. A period shorter than this falls within the time in which a depancreatized dog can live without ingesting either raw pancreas or lecithin supplements or choline supplements.

It is now well known that depancreatized dogs maintained under the dietary conditions of the present investigation are not in all respects normal. The eyes of both DA and DC show extensive bilateral cataractous formations.[12] It was found in this laboratory that lenticular opacifications may appear as early as one year after pancreatectomy. The blood lipids are markedly disturbed, a reduction in all lipid constituents, and in particular cholesterol esters, occurring soon after pancreatectomy.[8] The livers of these animals show enormous accumulations of lipids,[11] a change that may make its appearance as early as 10 weeks after pancreatectomy despite the insulin treatment. Nevertheless 2 facts must be noted about depancreatized dogs:

1. Survival for as long as 4.5 years is possible in the presence of these changes in liver, blood and lenses, provided such a dog is maintained on a diet containing meat, sucrose, bone ash and the necessary vitamins.

2. Judging by weight and subcutaneous fat, dogs DA and DC are in good nutritional state despite the pathological changes in the

[11] Kaplan, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1935, **108**, 201.

[12] Chaikoff, I. L., and Lachman, G. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 237.

3 tissues listed above. Except for the first week or two following pancreatectomy, during which time their appetites were somewhat poor, both dogs have had voracious appetites throughout their stay in the laboratory. Both dogs are still alive and cannot be distinguished from normal dogs in appearance or reactions.

8313 C

Measurement of Reagin in Non-Syphilitic Sera.*

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The reporting of serological tests for syphilis as positive or negative implies a qualitative difference between the bloods of syphilitic and non-syphilitic individuals. This concept assumes the presence in the serum of syphilitic patients of a substance designated as "reagin", which is lacking in patients without syphilis. In syphilitic patients, treated or untreated, it is highly probable that small amounts of reagin persist even though the serum is negative to the usual tests. As yet there is no evidence that reagin may not also be present in non-syphilitic sera.

If reagin is to be demonstrated in non-syphilitic sera, it is necessary to use a test so sensitive as to be invariably positive, to concentrate the serum, or to increase the reagin content to a point where it can be detected by the usual tests. We have chosen the last method, adapted from the work of Schreus and Foerster[1] who studied the Wassermann reaction in syphilitic patients treated to seronegativity. By adding to the sera of these patients subthreshold amounts of positive sera, these workers were able to obtain positive reactions. In place of the Wassermann we have substituted the Kline test because of its simplicity.

Suspensions are prepared from standard Kline antigen in the usual way. A stock reagin solution is prepared from positive serum after inactivation for 15 minutes at 56°C. Double precipitation is

* This work has been aided by a grant from the Rockefeller Fluid Research Fund.

[1] Schreus, H., and Foerster, R., *Z. f. Immunitäforsch. u. Exp. Therap.*, 1934, **82**, 53.