transplants were removed at the end of 12 weeks. In the remaining host the centrifuged bit, as already stated, had grown into a flat piece of cartilage and bone which measured 1.5 by 0.7 cm.

The most significant result observed from the foregoing experiments was the persistence with which cells distorted by violent centrifugalization regained and maintained their usual characteristics. The abnormalities which appeared are probably interpretable as normal tissues developing in unusual locations rather than as the result of fundamental changes induced in the constituent cells by centrifugalization. The host seemed to be merely a nutritional matrix for the centrifuged tissue which developed along the path of its original constitutional trend.

Summary. The cells of carcinoma tissue, after 30 minutes of centrifugalization in an ultracentrifuge at a displacement pull of 400,000 times that of gravity, show little trace of stratification of contents. Apparently the cytoplasm of such cells is of the consistency of a stiff gel. Such cells centrifuged for 20 minutes grew as readily as non-centrifuged cancer cells when implanted in young rats. The cells in bits of embryonic body-wall centrifuged for 12 and for 20 minutes respectively, although having their contents markedly stratified by the treatment, resumed growth when implanted subcutaneously in young rats, developing usually into hair-filled cysts of skin but occasionally into cartilage and bone. These results were probably due to the misplacement of embryonic tissues rather than to changes induced by centrifugalization.

9021 C

Retarded and Prolonged Action of Insulin Precipitated by Safranin.

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The injection into man and animals of a suspension of insulin precipitated by a protamine has been shown to lower the blood sugar for a period several times as long as that given by ordinary insulin.[1] The appearance of an article by Walker[2] on the use of dyes to pre-

[1] Hagedorn, H. C., Jensen, B. N., Krarup, N. B., and Wodstrup, I., *J. Am. Med. Assn.*, 1936, **106**, 177; Root, H. F., White, P., Marble, A., and Stotz, E. H., *J. Am. Med. Assn.*, 1936, **106**, 180.

[2] Walker, A. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 726.

cipitate proteolytic enzymes and bacteriophage led to the idea that such precipitants might be effective, when added to commercial insulin, in producing a suspension which would behave like protamine insulin.

Accordingly, a preliminary experiment was undertaken using equal parts of (1) U-40 insulin and (2) a 0.5% water solution of safranin O. After standing for 2 days at room temperature the mixture yielded a small amount of granular precipitate. The addition of one drop of 0.1 N NaOH produced an immediate flocculation which subsequently disintegrated into finer particles, leaving the solution turbid. By means of a series of phosphate buffer mixtures[3] it was found that precipitation occurred almost equally well at all pH values between 7.0 and 8.0. Since, however, insulin decomposes rapidly in alkaline solutions, all subsequent precipitations were carried out in as nearly neutral a medium as possible—namely, at pH 7.2.

Animal Experiments. The activity of the precipitated material was tried on one dog which was normal except for distemper and on one depancreatized dog.

(1) "Normal" dog, weight approximately 10 kg. The safranin precipitate from 5 cc. of U-40 insulin was washed once with water, added to the precipitates obtained during the experiments with the phosphate buffers, the whole redissolved in 6 cc. of a mixture of 1 volume of 0.1 N HCl and 2 volumes of 1/15 M KH_2PO_4, and injected intravenously after the animal had fasted for 19 hours. Determinations of the blood sugar showed the development of hypoglycemia which persisted for at least 7 hours.

(2) Depancreatized dog, weight 11 kg. Complete pancreatectomy had been performed 8 months previously and the animal had been maintained constantly on a weighed diet, including raw pancreas, and 18 units of insulin twice daily. In this series of experiments it was desired to compare the effects of (a) safranin insulin suspension, (b) the redissolved precipitate of safranin insulin, (c) protamine insulin suspension, (d) the redissolved precipitate of protamine insulin, and (e) regular insulin.

The safranin insulin suspension was prepared by adding 0.5 cc. of U-40 insulin to 0.5 cc. of saturated safranin O solution in a phosphate buffer of pH 7.2. The safranin insulin precipitate was obtained by a similar procedure, following which the mixture was centrifugated, the supernatant fluid poured off and drained, and the

[3] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Williams and Wilkins Co., Baltimore, 1932, vol. II, p. 816.

residue dissolved in 1 cc. of acid buffer (1 volume of 0.1 N HCl to 2 volumes of 1/15 M KH_2PO_4). The protamine insulin suspension was made according to directions and from materials supplied by Eli Lilly and Company. One cubic centimeter of buffered protamine solution containing a small amount of zinc is added to 4 cc. of U-50 insulin, resulting in 5 cc. of U-40 protamine insulin. One-half cubic centimeter of such a suspension was used for injection. The protamine insulin precipitate was obtained by centrifugating 0.5 cc. of the standard suspension, pouring off the supernatant fluid, wiping the sides of the tube dry and redissolving the residue in 1 cc. of the acid buffer solution described above.

Thus, in each experiment in which the suspension was used, the material to be injected was made up to contain 20 units of insulin. In each case in which the redissolved precipitate was employed, the amount injected was the amount precipitated from 20 units of insulin, and, since the precipitation of insulin is probably incomplete, it presumably contained less than 20 units. In the experiment in which regular insulin was used the dose was 20 units.

At intervals of one week or more one of the test substances was injected subcutaneously after the animal had been deprived of food and insulin for 24 hours. Blood for sugar determination was withdrawn from the external jugular vein immediately before and at intervals of 2 hours after the injection until 12 hours had elapsed. The final sample was taken the following morning before the administration of food and insulin and approximately 24 hours after the injection of the test substance. Analyses of the blood for sugar were performed by the Shaffer-Hartmann method[4] on unlaked blood filtrates made according to Folin.[5]

The results, shown in the accompanying table and chart, indicate that safranin insulin suspension rather closely resembles protamine insulin suspension in its ability to lower the blood sugar gradually and to maintain hypoglycemia for at least 12 hours, though the latter is somewhat more effective in both respects. Since the redissolved precipitates presumably contained less than 20 units of insulin, it is not surprising that the duration of their action was shorter than that of the suspensions; it was also shorter than that of 20 units of regular insulin. The redissolved protamine insulin precipitate reduced the blood sugar at almost exactly the same slow rate as did the safranin insulin suspension up to and including the 6-hour period, beyond which the latter was distinctly more effective in

[4] Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, 1921, **45**, 365.

[5] Folin, O., *J. Biol. Chem.*, 1930, **86**, 173.

TABLE I.

Effect of Subcutaneous Injection of Suspensions, Precipitates and Solution of Insulin on the Blood Sugar of a Depancreatized Dog.

Hours after injection	Blood sugar in mg. per 100 cc. after injection of				
	(a)	(b)	(c)	(d)	(e)
0	361	413	387	324	390
2	143	43	166	141	48
4	21	—	33	21	18
6	26	42	25	26	16
8	19	31	25	27	30
10	26	92	18	54	46
12	37	182	18	129	107
±24	351	334	293	348	—

(a) = 20 units safranin insulin suspension.
(b) = Redissolved precipitate from 20 units insulin precipitated by safranin.
(c) = 20 units protamine insulin suspension.
(d) = Redissolved precipitate from 20 units insulin precipitated by protamine.
(e) = 20 units regular insulin.

maintaining hypoglycemia. The redissolved safranin insulin precipitate, on the other hand, for reasons which are not clear, produced a rapid and brief lowering of the blood sugar, simulating the action of regular insulin.

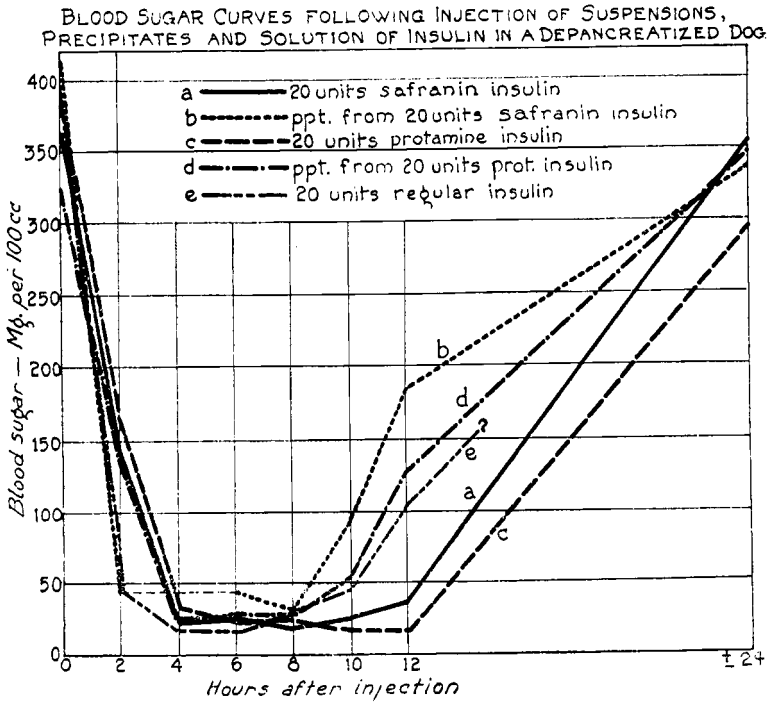

FIG. 1.

Summary. It has been shown that the addition of a weakly alkaline, buffered solution of safranin to a solution of commercial insulin yields an insulin-containing precipitate which, when injected in suspension into animals, causes hypoglycemia of gradual onset and extended duration. The blood sugar curve so produced is similar to, but not quite so depressed as, that given by the injection of an equal amount of protamine insulin suspension. The redissolved precipitates of safranin and protamine insulin are considerably inferior to the suspensions in retarding the fall of the blood sugar and/or prolonging the period of hypoglycemia.

9022 C

Bactericidal Effects of Vapors from Crushed Garlic on *Mycobacterium leprae.*

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Walton, Herbold, and Lindegren[1] showed that the vapors from freshly crushed garlic were germicidal to certain organisms. The present paper reports the effects of these vapors on different strains of acid-fast and non-acid fast *Mycobacterium leprae.*

Petri dishes containing 3% glycerin nutrient agar were warmed in an incubator at 37.5°C. for about 2 hours and a heavy suspension of organisms was then spread on the agar. After inoculation, one gram of garlic, freshly ground in a meat-chopper, was placed on the inverted cover of the petri dish below (but not in contact with) the agar. The dish was sealed with a large rubber band and placed in the incubator at 37.5°C. The fumes from the garlic were allowed to fill the air-space below the agar surface. The amount of volatile substances transported to the agar was varied by exposing the agar to the fumes of the garlic for different lengths of time. Intervals of from one minute to 2 hours were used. At the end of each interval, the dish was removed from the incubator and the cover containing the garlic replaced by a sterile cover. Then the dish was returned to the incubator and after 3 days' incubation the amounts of growth on the various plates were compared (Table I). The heaviest growth was given a score of 4. If the treated plates

[1]Walton, L., Herbold, M., and Lindegren, C. C., *Food Research,* 1936, **1**, 163.