tration of compounds I to VI caused no pancreatic damage requires the following comment:

(a) The blood sugars were frequently above the normal level and this has been attributed to the technique used. When normal rabbits were handled in this manner blood sugars have been of this magnitude. There is a clear distinction between these values and those of rabbits with alloxan diabetes in which the blood sugars are characteristically high *i.e.*, 300 to 500 mg per 100 cc. The consistently negative urines provided a mass of evidence, not included in the tables, that these rabbits were not diabetic as a result of the quinoline compounds.

(b) The conclusion that these compounds are not diabetogenic is based on results in a single species. Although the rabbit was used by Sheehan[1] in the study of styryl quinoline, and although the rabbit is the animal most susceptible to alloxan, this limitation must be mentioned.

(c) The tendency to compare other toxic chemicals with alloxan may lead to errors in spite of every effort that has been made to observe diabetogenic activity. Thus, alloxan in similar sublethal doses would have caused diabetes with certainty, within the time limits of these experiments. A more insidious toxin could theoretically escape detection.

As far as we know, the results obtained with compound VII provide the first confirmation of Sheehan's[1] report that styryl quinoline caused injury of the pancreatic islands. As we gave styryl quinoline to only 4 animals our negative results with this compound (VI) might be accidental. This series was small because of Bailey's personal communication that he had not produced lesions of the islands or diabetes with styryl quinoline. The presence of lesions in the larger series of animals given a derivative of styryl quinoline (VII) is in general accord with Sheehan's[1] observations. He described "from memory" the consistent occurrence of islet necrosis after lethal doses of styryl quinoline (VI). In our series lesions occurred in the islets only after lethal doses but their occurrence was quite inconstant. In view of the absence of detailed protocols in the original report[1] further discussion is omitted.

Summary. Six quinoline derivatives have been administered intravenously and subcutaneously to rabbits and rats. There was no functional or anatomical evidence of damage to the islands of Langerhans or to any other organ under the conditions employed.

A seventh compound, N- 2-(p-Diethylaminostyryl)-6-methyl quinoline-ethochloride caused lesions of the islands resembling the early necrosis produced by alloxan. The lesions occurred in 8 of 28 animals tested. Islet necrosis was always associated with lethal dosage but death from the drug was not always accompanied by necrosis. Tubular damage in the kidney was found in all cases examined. Survival with diabetes was not observed.

16843

Potential Antigenicity of Parenterally Administered Cytochrome C.

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The separation and purification of cytochrome C by Keilin *et al.*[1] opened the field for its use in animal and human experimentation, in which encouraging results have since been claimed.[2-4] Its production usually involves

[1] Keilin, D., Hartree, E. F., and Hartree, F. R. S., *Proc. Roy. Soc. Med.*, 1937, **122**, 299.

[2] Proger, S., *Bull. New England Med. Center*, 1945, **7**, 1.

[3] Proger, S., and Decaneas, D. J., *Bull. New England Med. Center*, 1945, **7**, 149.

[4] Frank, H. A., Seligman, A. M., and Fine, J., *J. Clin. Invest.*, 1945, **24**, 435.

extraction from horse or beef heart tissue, but the possibility of antigenicity has not been noted in any of the papers on cytochrome C seen to date, and only one brochure with a commercially available preparation suggests such a possibility.

Experimental. Three preparations of cytochrome C of different manufacturers were obtained; two were of equine origin, and one was of mixed bovine and equine origin. (For convenience, these preparations hereafter will be referred to respectively as CC-I, CC-II, and CC-III). All sensitizing doses were intraperitoneal. All shocking doses were via the dorsal penile vein.

Fourteen male guinea pigs were given 3 successive sensitizing injections of CC-I intraperitoneally at 2-3 day intervals. Group I, (9 animals), received 1 mg per dose, and Group II, (5 animals), received 5 mg per dose. In Group I, 3 weeks after the last sensitizing injection, 6 were challenged by the injection of CC-I via the dorsal penile vein. There was one fatal anaphylactic shock, 4 reactions with recovery, and one negative result. One week later the 3 of this group not previously tested were similarly challenged, and all died of anaphylactic shock. In Group II, all 3 animals challenged at 3 weeks died of anaphylactic shock, as did also the remaining 2 challenged at 4 weeks.

In a second experimental series, giving only 2 sensitizing injections of 2 mg per dose, 6 animals were sensitized to CC-I and 6 received similar doses of CC-II. Nineteen days later each group was divided so that 3 received the original sensitizing material, and the other 3 received the alternate product. None of the animals sensitized to CC-I showed any reactions, either to CC-I or CC-II. On the other hand, all animals initially sensitized to CC-II exhibited some degree of anaphylaxis when challenged with either CC-I or CC-II, and one death occurred with each material.

A third series of 9 guinea pigs was sensitized with CC-III, in 2 successive doses of 2.5 mg each. One month later 4 were challenged with the same material, 3 mg intravenously. None showed any shock symptoms. The remaining animals were challenged 5½ weeks after the last sensitizing dose, and none of these showed any reactions. Those tested at one month were again challenged 10 days later, and again no reactions were observed.

That the reactions observed in the first two series are due to primary antigenicity and not to a possible horse serum contamination was shown in a control series of 7 guinea pigs sensitized to horse serum. One animal given 0.75 cc horse serum died of anaphylactic shock. The 6 others received CC-I in doses from 3 to 10 mg and none showed any symptoms of shock. Exhaustion of the limited supply of the same lots of cytochrome C made it impossible to pursue further reciprocal testing with horse serum.

Discussion. The above experiments show that the clinical application of cytochrome C may be attended by a hazard similar to that experienced with horse serum. Attention should be drawn to some of the variations noted in the above experiments. Anaphylactic symptoms were more frequent and severe when the sensitizing doses were multiple and large, and when the incubation period was prolonged. When the number or quantity of sensitizing doses was reduced, anaphylactic symptoms diminished or were absent, so that no antigenicity was demonstrated under the experimental conditions. However, we have shown[5] that weakly antigenic materials, given a greater number of times for sensitization, and allowed a longer incubation period, may be shown to possess antigenicity not demonstrable by more commonly employed methods.

The possibility of a serious or even fatal anaphylactic reaction in the potentially sensitive human patient must therefore be considered when cytochrome C is administered intravenously, particularly if more than once, and testing precautions should be taken.

Summary. Guinea pigs sensitized with cytochrome C of equine origin when challenged with the same material, were shown to exhibit anaphylactic reactions, ranging to fatal, in a relatively large percentage of animals. Cross sensitivity could not be demonstrated between cytochrome C and horse

[5] Roth, L. W., Richards, R. K., and Shepperd, I. M., *Fed. Proc.*, 1948, **7**, 105.

serum, but remains a possibility. Cross sensitivity to cytochrome C of different manufacture was demonstrated. The possible hazards of its use clinically are emphasized.

16844

Assay of Anti-Pernicious Anemia Factor with *Euglena.*

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It was noted that massive growth of the algal flagellate *Euglena gracilis* depended on unknown growth factors present in crude casein but absent in certain plant proteins such as edestin and concanavallin A.[1] This factor was removed from casein by repeated isoelectric precipitation. It was subsequently found that alcoholic extracts of crude casein‡ were active, and refined liver extract was recently reported to possess considerable activity.[2] It was found in the present investigation that this growth factor requirement was satisfied by a combination of crystalline anti-pernicious anemia factor (APA)[3] plus thiamine. These findings became the basis of an assay method for APA.

Trial of APA was suggested by the good agreement between the animal protein factor (APF) activity as measured with chicks, and the growth-promoting effect for *Euglena* of injectable liver extracts and of microbial APF concentrates. The latter were found to produce an hematopoietic response in pernicious anemia.[4]

Experimental. The organism used was *Euglena gracilis* var. *bacillaris.* The culture vessels were exposed to "daylight" fluorescent lamps at 25° to 31°C. The optimal temperature was 28° to 31°C; inhibition effects began to appear at approximately 32°C. At first, assays were carried out in 25-ml Erlenmeyer flasks covered with glass caps, and containing 10 ml of medium. It was later found convenient in routine assays to employ 100 x 13 mm tubes containing 2 ml of medium, illuminated from below. The light was supplied by 4, 40-watt lamps mounted side by side, 30 cm from the cultures. Light intensity did not appear to be a limiting factor at the levels of growth reported here. The basal medium is shown in Table I. The thiamine requirement was approximately 0.5 mμg/ml for half

TABLE I.
Composition of Basal Medium.

	Per 1 final medium (pH 6.5)	
$NH_4H_2PO_4$	0.8	g
Potassium citrate, monohydrate	0.2	"
$MgSO_4 \cdot 7H_2O$	0.2	"
Sodium butyrate	2.0	"
Monosodium glutamate	1.0	"
$CaCl_2$	0.1	"
$FeSO_4 \cdot 7H_2O$	20	mg
$MnSO_4 \cdot H_2O$	6	"
$CoSO_4 \cdot 7H_2O$	5	"
$ZnCl_2$	0.8	"
$Na_2MoO_4 \cdot 2H_2O$	1.0	"
$CuSO_4 \cdot 5H_2O$	0.08	"
Thiamine chloride	0.1	"

* Haskins Laboratories. Aided by a grant from Lederle Laboratories Division, American Cyanamid Co.

† Lederle Laboratories Division, American Cyanamid Co.

1 Hutner, S. H., *Arch. Protistenk.*, 1936, **88**, 93.

‡ Generously supplied by the Nutritional Biochemicals Corporation, Cleveland, Ohio.

2 Provasoli, L., Hutner, S. H., and Schatz, A., PROC. SOC. EXP. BIOL. AND MED., in press.

3 Smith, E. L., *Nature*, 1948, **161**, 638. A sample was kindly furnished by the Glaxo Laboratories, Ltd.

4 Stokstad, E. L. R., Page, A., Pierce, J., Franklin, A. L., Jukes, T. H., Heinle, R. W., Epstein, M., and Welch, A. D., *J. Lab. Clin. Med.*, 1948, **33**, 860.