TABLE I.
Data on Blood Pressures of Anesthetized, Trained and Untrained Dogs, Obtained by Direct (Arterial Puncture) and Indirect (Tail Plethysmograph) Means.

Dog	Anesthesia	Trained	Blood pressure, mm of mercury		
			Plethysmograph	Femoral artery	Difference
1	+	+	140	135	5
	—	+	140	130	10
	—	+	146	136	10
	+	+	132	126	6
	+	+	132	126	6
2	+	—	175	168	7
3	+	—	170	162	8
	+	—	138	132	6
	—	—	175	132	43
4	—	—	185	145	40
	+	—	142	134	8
5	+	—	118	110	8
	+	—	146	140	6
	+	—	118	112	6

and in well-trained dogs. It was found that inflation of the cuff sometimes caused certain dogs to contract the caudal muscles enough to give blood pressure values that were too high. In such animals local caudal or spinal block prevented this from occurring. The data obtained with the tail plethysmograph were simultaneously checked against values obtained with pressures taken by direct arterial puncture and mercury manometer. Representative data on the blood pressure obtained from trained and untrained dogs, with and without anesthesia, are given in Table I. The blood pressure of the anesthetized dog taken by the plethysmograph agreed very well with that obtained by direct arterial puncture but was, on an average, 6 to 8 mm of mercury higher, while in the unanesthetized trained dog the blood pressure was about 10 mm higher. In the unanesthetized untrained animal differences of as much as 40 mm of mercury were observed. One must, however, keep in mind the rapid vasomotor alterations of such animals to outside stimuli and recognize that changes of this magnitude may occur normally. A comparison in anesthetized animals of the response of the blood pressure obtained simultaneously with the tail plethysmograph and a mercury manometer following intravenous injections of sodium nitrite, ephedrine and pitressin showed values that differed by only a few millimeters of mercury.

Summary. The tail plethysmograph as used on the rat has been modified for use on the dog. When properly applied, it affords an easy and repeatable measure of arterial blood pressure in close agreement with values obtained by the mercury manometer.

14914

Nucleic Acid Derivatives as Growth Factors for Certain Group A Hemolytic Streptococci.*

ARMINE T. WILSON. (Introduced by A. E. Mirsky.)
From the National Naval Medical School, Bethesda, Md.

The development of a satisfactory test for the *in vitro* resistance of Group A (Lancefield) streptococci to sulfonamides has been hampered by the lack of a medium which would support adequate growth of freshly isolated strains and would, at the same time, contain little or no sulfonamide antagonists. It was

* The opinions expressed in this paper are those of the author and do not necessarily represent those of the Navy Department.

discovered that the semi-synthetic medium of Roe and Adams[1] fulfilled these desiderata when it was supplemented with 10% normal rabbit serum, and such a medium was made the basis of a method for testing resistance of streptococci to sulfonamides.[2] It was discovered, however, that certain strains which grew well in this medium failed to grow at all when horse serum was substituted for rabbit serum. Other strains grew well whichever serum was used.

The composition of the Roe and Adams medium has been given elsewhere in detail.[2] It consists of caseine hydrolysate, cystine, tryptophane, dextrose, mineral salts, biotin, pyridoxin, nicotinic acid, calcium pantothenate, thiamine, riboflavine, adenine sulfate, uracil, glutamine, asparagine, choline, thioglycollic acid and buffer salts.

There appeared to be two possible explanations for the failure or horse serum to support growth when it was added to this medium: either it exerted an inhibitory influence on certain Group A strains; or it lacked one or several factors essential to the growth of these strains.

That the former explanation was not the case was shown by the failure of horse serum to have an inhibitory effect when added in concentrations as high as 25% to a mixture of the semi-synthetic medium and the smallest amount of rabbit serum (3%) which would produce maximal growth in the absence of horse serum.

It appeared, therefore, that certain strains of Group A streptococcus required a factor for growth which was lacking in horse serum but which was supplied by rabbit serum. For convenience this was called the RS factor.

The work reported here presents evidence concerning the dependence of various Group A streptococci on the RS factor, the distribution of the factor in certain biological materials and the ability of certain nucleic acid derivatives to replace the factor.

TABLE I.
Dependence of Various Group A Streptococci on the RS Factor According to Serological Type.

Type	No. requiring RS factor	No. not requiring RS factor
1	3	1
2	0	4
3	0	4
4	1	4
5	3	1
6	1	2
10	0	1
11	1	0
13	0	2
14	1	1
17	0	5
18	0	3
19	8	12
23	1	0
24	0	1
26	0	4
28	0	1
29	2	1
30	4	2
All types	25	49

To eliminate the chance that the absence of the factor was a peculiarity of the particular horse which served as a source of serum for these experiments, sera from 5 other horses† were tested and all were found to be similar in their lack of the factor.

TABLE II.
Relationship of RS Requirement and Type Antigens of Type 19 Streptococci.

	No. requiring RS factor	No. not requiring RS factor
Strains containing M and T antigens of Type 19	7	8
Strains containing M antigen but no T antigen of Type 19	1	4

Further studies showed that human, pig, dog and sheep sera resembled rabbit serum in supplying the factor, whereas cow, rat and mouse sera lacked it.

For testing the ability of the various sera to support growth of the exacting strains, tubes containing 2 cc of a medium composed of 90% semi-synthetic medium and 10% of the serum

[1] Roe, A. S., and Adams, M. H., abstracted in *J. Bact.*, 1944, **47**, 432.

The present author wishes to express his appreciation for permission to use this medium before full details of its composition have been published by them.

[2] Wilson, A. T., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 130.

† These sera were kindly furnished by Dr. L. Earl Arnow of Sharp and Dohme, Inc., Glenolden, Pa.

to be tested were given inocula of approximately 10^{-5} cc of strains known to require the factor by the technique of MacLeod and Mirick,[3] and were grown at 37° for 24 hr. Control tubes were set up using rabbit serum in place of the unknown sera. Growth was estimated visually and maximal growth compared favorably to that obtained with a very rich medium such as Todd-Hewitt broth.

Peptone and fresh meat infusion from beef and horse muscle were shown to contain the factor in large amounts.

A number of substances which had previously been identified as growth factors for various microorganisms, but which were absent from the Roe and Adams medium, were added individually to the deficient horse serum-semi-synthetic medium and it was discovered that the addition of xanthine in concentrations as low as 0.6 γ per cc was effective in promoting growth of the exacting strains.

Since adenine and uracil were already present in the semi-synthetic part of the medium, it appeared that the need for xanthine represented a specific, or at least an additional, purine requirement.

A survey of representative strains of various serological types of Group A streptococcus‡ was undertaken in order to determine the frequency of dependence on the RS factor. This dependence was determined by inoculating 10^{-5} cc of blood broth culture into each of 2 tubes which contained respectively 2 cc of a mixture of 90% semi-synthetic medium with 10% horse serum, and 2 cc of the same mixture with xanthine added to a final concentration of 1 mg per 100 cc. Most of the strains could be sharply divided into two classes: those which grew equally well in both media and those which grew well in the xanthine-containing medium but gave no visible growth in the medium to which no xanthine had been added, even after incubation as long as seven days. A few strains grew very slowly in the xanthine-free medium, but final growth, after several days, was equal to that in the medium containing xanthine.

Approximately one-third of the strains required the RS factor (Table I). The number of strains tested for most of the types was small, but the frequent occurrence of strains of both classes within the same type makes it unlikely that there is any association between this requirement and serological type. Studies of strains of Type 19 which had been analyzed for their M- and T-antigens by Dr. Lancefield, and which were kindly supplied by her, showed no correlation between xanthine requirement and the presence of either of these antigens (Table II). It was further shown that there was no association between resistance of a strain to sulfonamide and its dependence on the factor.

No instance was encountered of an exacting strain changing to a non-exacting strain or vice versa. Occasionally strains which were known to be exacting grew out unexpectedly in the deficient medium, but repetition of the experi-

TABLE III.
Ability of Certain Materials to Replace the RS Factor.

Materials having RS factor effect	Materials not having RS factor effect
Xanthine[1]	Adenine[1]
Guanine[1]	Uracil[1]
Hypoxanthine[2]	Cytosine[2]
Guanosine[2]	Thymine[2]
Desoxyguanosine[2]	Uric Acid
Adenosine[2]	Caffeine
Yeast nucleic acid[3]	Alloxan[6]
Fresh beef infusion	Horse serum
Fresh horse infusion	Cow "
Peptone	Rat "
Rabbit serum	Mouse "
Human "	Streptogenin[7]
Pig "	Thymus nucleic acid[4]
Dog "	
Sheep "	
Thymus nucleic acid[4] after degradation by desoxyribonuclease.[5]	

[1] Eastman Kodak Co., Rochester, New York.

[2] From the P. A. Levene Collection of the Rockefeller Institute through the courtesy of Mr. E. B. Smith.

[3] City Chemical Co., New York.

[4] From Dr. A. E. Mirsky of the Rockefeller Institute, New York.

[5] From Dr. M. McCarty of the Rockefeller Institute Hospital, New York.

[6] From Dr. M. Pijoan, Naval Research Institute, Bethesda, Md.

[7] From Dr. D. W. Woolley, of the Rockefeller Institute, New York.

[3] MacLeod, C. M., and Mirick, G. S., *J. Bact.*, 1942, **44**, 277.

‡ These strains were generously supplied by Dr. Rebecca C. Lancefield of the Rockefeller Institute Hospital, New York.

TABLE IV.
Effect of Desoxyribonucleic Acid Before and After Treatment with Desoxyribonuclease.

	Strains known to require the RS factor			Strains known not to require the RS factor		
	2848F	2884F	2885F	859GL	861GL	Uninoculated
Desoxyribonucleic acid alone	—	—	—	++++	++++	—
Desoxyribonucleic acid with desoxyribonuclease	++++	++++	++++	++++	++++	—
Desoxyribonuclease alone	—	—	—	++++	++++	—
Inactivated desoxyribonuclease alone	—	—	—	++++	++++	—
Desoxyribonucleic acid with inactivated desoxyribonuclease	—	—	—	++++	++++	—
Neither	—	—	—	++++	++++	—

ment showed that this was due to some unrecognized variation in the experimental circumstances and not to a change in the requirements of the organism. No serious attempt has been made to "train" exacting strains to grow in the absence of the RS factor.

Various other nucleic acid derivatives were tested for their ability to replace xanthine as the growth factor. This was done by adding solutions containing 25 or 100 mg of the test substances to the deficient medium in such quantity that their final concentration in the medium was 1 mg per 100 cc. Controls using the deficient medium alone and the deficient medium with 1 mg of xanthine per 100 cc were used. The tubes were inoculated with 10^{-5} cc of culture of several strains known to require the RS factor, and, as an additional control, similar tubes were inoculated with strains known not to require the RS factor.

It was found that guanine, hypoxanthine, desoxyguanosine, adenosine and a commercial preparation of yeast nucleic acid were active. On the other hand cytosine, thymine, uric acid, caffeine, alloxan and thymus nucleic acid were inactive (Table III). Adenine and uracil were known to be inactive, since they were already present in the semi-synthetic part of the medium.

Quantitative studies of the relative effectiveness of the various derivatives showed, rather surprisingly, that those which had any activity at all were approximately equal in effect, weight for weight, regardless of molecule size. Too much emphasis should not be placed on these quantitative studies since the medium is not completely defined. Small quantities of unknown active substances or of antagonists may be contributed by the serum, and these unknown materials may not bear a uniform relationship to the various derivatives studied.

It is likely that the ribonucleic acid preparation which was used contained small quantities of free nucleotides and nucleosides and its activity may have been due to the presence of these substances. Possible explanations of the contrasting ineffectiveness of desoxyribonucleic acid were that it was uncontaminated with nucleotides and nucleosides, that desoxyribose could not replace ribose in an active molecule or that the more highly polymerized state of the thymus preparation rendered it unavailable as the factor. The effectiveness of desoxyguanosine indicated that the nature of the sugar did not determine activity. When thymus nucleic acid was exposed to the action of desoxyribonuclease it was converted to an active compound (Table IV). It is believed that the effect of this enzyme leads to a degradation no greater than depolymerization.[4] The enzyme preparation itself was inactive as the RS factor, and was unable to convert thymus nucleic acid to an active compound after exposure to a temperature of 60° for 30 minutes.

A preparation of streptogenin, known to be a growth factor for certain streptococci and

[4] McCarty, M., personal communication.

supplied by Dr. D. W. Woolley, was inactive.

Discussion. The effects of nucleic acid derivatives on the growth of various microorganisms have been studied extensively in recent years.[5-17] In the majority of instances the experimental conditions have been such that all growth requirements of the strains could be met by materials of known chemical composition, and the effects of varying the individual constituents could be studied with great precision. Unfortunately in the case of group A streptococci no fully defined medium exists which will grow all strains luxuriantly. Pappenheimer and Hottle[7] made some interesting observations on one Group A strain which they were able to grow in a semisynthetic medium. This medium differed from the medium of Roe and Adams chiefly in its use of gelatin hydrolysate in place of caseine hydrolysate, in its lack of choline and asparagine, and in its content of added tyrosine and glutathione. The strain they used, C203S, required adenine for growth at atmospheric CO_2 tension but not at increased CO_2 tension. Uracil accelerated the rate of growth but was not essential. They found that adenine could be replaced by guanine, guanosine, guanylic acid, xanthine or hypoxanthine. This strain was tested by the technique presented in this paper and was found not to be an exacting strain with respect to the RS factor.

Although it is likely that xanthine and its equivalents act as growth factors, the fact that these observations have been made in a complex serum-containing medium makes it difficult to be certain of this point. It has been shown[13] that the various purines and pyrimidines may be mutually antagonistic in relation to growth stimulation, and this antagonism has been compared to that existing between para-amino-benzoic acid and sulfonamides, between pantoyl taurine and pantothenic acid, and between pyridine 3 sulfonic acid and nicotinic acid. It is conceivable that xanthine and its equivalents serve merely to counteract the action of some unidentified antagonistic substance which is present in horse serum but not in rabbit serum. Elucidation of this point and particularly of the quantitative relationships of the various derivatives must await the development of a chemically defined medium which will grow Group A streptococci.

In view of the observations of Loring and Pierce[16] that nucleosides and nucleotides were many times more active than their constituent pyrimidines for growth stimulation of *Neurospora* mutants, it is interesting to note that adenosine was effective as the RS factor whereas adenine itself was not. It is hoped that this observation can be repeated with other preparations of adenosine and with adenylic acid.

The ability to divide Group A streptococci of a single serological type into two classes on the basis of their dependence on the RS factor may place another tool in the hands of epidemiologists who find it desirable to "tag" organisms.

Conclusions. 1. Approximately one third of a group of representative Group A streptococci required a factor for growth in a semisynthetic medium reinforced with serum which was present in rabbit, human, pig, dog and sheep sera. This factor was absent from horse, cow, rat and mouse sera. 2. The factor could be replaced by yeast nucleic acid and certain of its derivatives, such as xanthine, guanine and hypoxanthine. It could not be replaced by certain other derivatives such as

5 Richardson, G. M., *Biochem. J.*, 1936, **30**, 2184.

6 Moller, E. F., *Z. Physiol. Chem.*, 1939, **260**, 246.

7 Pappenheimer, A. M., Jr., and Hottle, G. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 645.

8 Snell, E. E., and Mitchell, H. K., *Proc. Nat. Acad. Sci.*, 1941, **27**, 1.

9 Stokstad, E. L. R., *J. Biol. Chem.*, 1941, **139**, 475.

10 Mueller, J. H., and Miller, P. A., *J. Biol. Chem.*, 1941, **140**, 933.

11 Robbins, W. J., and Kavanagh, F., *Proc. Nat. Acad. Sci.*, 1942, **28**, 4.

12 Robbins, W. J., and Kavanagh, F., *Proc. Nat. Acad. Sci.*, 1942, **28**, 65.

13 Pennington, D. E., *Proc. Nat. Acad. Sci.*, 1942, **28**, 272.

14 Feeney, R. E., and Strong, F. M., *J. Am. Chem. Soc.*, 1942, **64**, 881.

15 Hopkins, F. G., and Simon-Reuss, I., *Proc. Royal Soc.*, Ser. B, 1944, **132**, 253.

16 Loring, H. S., and Pierce, J. G., *J. Biol. Chem.*, 1944, **153**, 61.

17 Gibert, E. B., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 363.

adenine and uracil. 3. Desoxyribonucleic acid was inactive as the factor, but became active after depolymerization by desoxyribonuclease. 4. The lack of uniformity of Group A streptococci with respect to dependence on this factor shows that nutritional studies performed on a single strain cannot be regarded as representative of the entire group.

Acknowledgement of indebtedness to the many individuals who have supplied materials for this investigation will be found in the text, in the footnotes and in the tables of this paper. A word is appropriate here concerning the generosity of those who have these rare materials in their possession and who lend them so freely. An undertaking of this sort would be impossible without it. The author wishes further to thank Dr. A. E. Mirsky for his interest and for his many suggestions.

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Diabetes in a Totally Depancreatized Man.*

HENRY T. RICKETTS, ALEXANDER BRUNSCHWIG AND KATHRYN KNOWLTON.

From the Departments of Medicine and Surgery and the Frank Billings Clinic, School of Medicine, University of Chicago.

We wish to report observations on the diabetes of a totally depancreatized man. The surgical aspects of the case have been described elsewhere.[1] The patient in question had had spontaneous diabetes, known for 9 months before he entered the hospital, and his father also had had the disease. His presenting complaints were abdominal pain and diarrhea of 2 years' duration and, recently, thirst, polyuria and loss of weight. The symptoms, except for the diarrhea, were largely ameliorated by diet and insulin. While under treatment he developed jaundice and at operation was found to have a carcinoma of the pancreas with infiltration of the neighboring organs. The entire pancreas, duodenum, stomach, spleen, left adrenal and most of the omentum were removed. The liver appeared normal. Histologic examination of the uninvolved head and neck of the pancreas (Dr. George Gomori) showed insular changes characteristic of diabetes. At the conclusion of the operation there was no gross evidence of remaining neoplasm. The postoperative course was uneventful and the patient was out of bed on the sixteenth day. For 6 weeks he was maintained on both protamine zinc and crystalline insulin, but thereafter, in order to facilitate studies, was given crystalline insulin alone. He also received vitamin preparations parenterally, atropine to control diarrhea, and at intervals large doses of powdered pancreas. He survived for 14 weeks after operation, death being due to recurrence of the carcinoma and, terminally, to the withdrawal of insulin.

Insulin Requirement. Before operation, on a diet of C 398, P 200, F 12 g (glucose equivalent 511 g), the amount of insulin necessary to keep the urine nearly sugar-free was from 40 to 65 units per day. The body weight at this time was 72 kg (158lb). Two months after operation, when the patient was up and about, the diet was C 401, P 102, F 11 g (glucose equivalent 459 g) and the insulin requirement was about 40 units per day. The body weight was now 56 kg (123 lb). The apparent reduction in the demand for insulin may have been due to 2 factors: first, the lower glucose value of the postoperative diet, and second, the loss of 16 kg of weight. The striking fact, however, is not that the insulin requirement was lower, but that it was no higher.

Sensitivity to insulin was marked, the patient showing frequent hypoglycemic reactions.

Withdrawal of Insulin. Approximately 3

* This study was aided by a grant from the Charles H. and Mary F. S. Worcester Memorial Fund of the University of Chicago.

1 Brunschwig, Alexander, and Ricketts, Henry T., *Surg. Gyn. and Ob.*, 1945, in press.