indicate that the chronic administration of this simple chemical compound interferes with a hitherto unrecognized process, perhaps by making unavailable a component of some enzyme system.

E. B. ASTWOOD
A. M. HUGHES
M. LUBIN
W. P. VANDERLAAN
R. D. ADAMS

DEPARTMENTS OF PHARMACOLOGY,
MEDICINE AND NEUROLOGY,
HARVARD MEDICAL SCHOOL

THE MECHANISM OF ACTION OF ALLOXAN ON BLOOD SUGAR

THE intravenous administration of alloxan induces a triphasic modification of the blood sugar level: (1) hyperglycemia; (2) hypoglycemia; (3) hyperglycemia. We have studied these phenomena in several species, particularly the chlorolosed dog (100 mg of alloxan/kg of body weight) and the toad *Bufo arenarum* Hensel (200 mg/kg).

The initial hyperglycemia did not appear in hepatectomized dogs or toads nor in eviscerated dogs. It was observed in adrenalectomized animals (5 dogs and 6 toads) and in 3 dogs with previous section of the splanchnic nerves (major and minor) so that it can not be attributed to either adrenaline or cortical hormones. It was also observed in recently hypophysectomized toads. If injected in the portal vein, alloxan produces a higher initial hyperglycemia (5 dogs) than if injected in the saphenous vein (8 dogs).

The secondary hypoglycemia is not due to liberation of insulin by the β cells of the islets undergoing destruction. Nine dogs totally depancreatized half an hour before injection of alloxan, showed a marked hypoglycemia beginning 1, 2, 2, 2, 2, 3, 3, 4 and 5 hours after injection; the blood sugar level reaching in 7 cases to 50 and 24 mg per 100 cc. Six of these dogs showed initial hyperglycemia. Pancreatectomized controls with no alloxan only in a few cases showed slight and brief diminution of the blood sugar level half an hour after the operation, followed by a gradual and steady increase from 2 to 6 hours after the operation, reaching at that time 0.149 and 0.180 g per 100 cc of blood.

In 7 dogs depancreatized 24 to 48 hours previous to the injection of alloxan there was no hypoglycemia: on the contrary, the blood sugar level was slightly increased. In only one case was there a moderate decrease (from 0.217 to 0.134 g per cent. between 5 to 6 hours after the injection).

In pancreatectomized toads, alloxan injected immediately after the operation either prevents or decreases the diabetic hyperglycemia during the next 24 hours. If injected 24 hours after pancreatectomy, the existing diabetic hyperglycemia decreases as shown by the blood samples 24 hours after injection. Alloxan also notably decreased the diabetogenic action of the *pars distalis* of the hypophysis when subcutaneously injected to the hypophysectomized and depancreatectomized toad.

The capacity of the pancreas to secrete insulin was investigated by grafting in the neck through vascular anastomosis the duodeno-pancreas of dogs to dogs rendered diabetic through pancreatectomy performed 24 hours before. Normal pancreas decreases the blood sugar to normal level within 3 to 5 hours. Pancreas from dogs injected with alloxan 24 hours (6 dogs) or 48 hours (2 dogs) before extraction and grafting did not secrete insulin in 4 cases, the secretion was very reduced in 3 cases and only in 1 was the secretion normal. It is interesting to note that the pancreas was taken in some cases from animals that were still hypoglycemic. The β cells of the islets showed lesions in all cases (Dr. Di Pietro).

The final rise of the blood sugar reaches sometimes (rats, rabbits and dogs) higher values to those usually observed after pancreatectomy. Values of 0.700 and 1.00 g per 100 cc of blood have been observed. Possibly the liver plays some part in this phenomenon.

Therefore: (1) The liver is essential for the initial hyperglycemia produced by alloxan. Hyperglycemia is observed in adrenalectomized animals and those with section of the splanchnic nerves. It must be attributed principally to a direct action of alloxan on the liver. (2) The secondary hypoglycemia is not due to liberation of insulin, but to an extrapancreatic effect: probably lack of glucose production by the liver. The liver of the animal already in a diabetic condition is generally insensible to this action of alloxan. (3) The final hyperglycemia is mainly due to the destruction of the β cells of the islets of Langerhans, and becomes permanent if the animal survives. (4) The liver plays an important role during the 3 phases of modification of blood sugar level.

B. A. HOUSSAY
O. ORÍAS
I. SARA

INSTITUTO DE BIOLOGÍA Y MEDICINA,
EXPERIMENTAL, COSTA RICA 4185,
BUENOS AIRES, ARGENTINA

THE PRODUCTION OF ANTI-PENICILLINASE IMMUNE SERA

IT is well known that the injection of an antigen (precipitinogen) parenterally in animals stimulates the production of antibodies (precipitins). In order that a substance may be precipitinogenic, it apparently must contain a soluble protein.[1]

[1] F. P. Gay and Associates, "Agents of Disease and Host Resistance," Charles C Thomas, Baltimore, Md., 1935.

The penicillinase prepared in our laboratories[2] for penicillin inactivation consists of about 5 per cent. protein. Since this preparation was not tolerated well by rabbits, it became necessary to prepare specially purified penicillinase[3] for this purpose. It was believed that immune sera could be prepared using this latter preparation of penicillinase (containing about 8 per cent. protein) as the antigen.

METHODS

Preliminary experiments with rabbits were conducted to determine the amount of antigen to be injected, the number of injections and the interval between the injections, which was necessary to induce the formation of the immune bodies. The diluted antigen was injected slowly in small, graded doses in order to prevent sensitization and associated reactions. The intravenous route was used since purified penicillinase was not very toxic for rabbits and there was little danger of eliciting anaphylactic responses by repeated injections. The injections of antigen were made in the marginal vein of the right ear.

Two series of injections were found to give reliable results. 1.0 mgm of protein per kilogram of body weight was given five times weekly for two weeks and then 2.0 mgm of protein per kilogram of body weight five times during the third week, bleeding was made daily from the marginal vein of the left ear. Prior to bleeding the animals were fasted overnight so as to avoid serum opalescence usually due to an increase in the lipoidal elements of the serum from digesting food.

When the serum from this preliminary bleeding was found to be sufficiently high in antibody titer (usually five to ten days after the last injection) the animal was anesthetized and bled from the heart. The blood was allowed to clot, then centrifuged and the serum pipetted from the top. The serum was then Seitz filtered and stored in the refrigerator to "age" for at least one week before subsequent testing for titer. This was done so as to remove any substances which may precipitate spontaneously. When any such non-specific materials precipitated, the serum was again Seitz filtered.

The precipitin test was performed using the so-called serum dilution method as described by Culbertson.[4] The tubes were incubated at 37° C for two hours to obtain practically complete precipitation[5] and then stored in the refrigerator overnight. The following were the maximum titers obtained after a three-week injection series (see Table 1).

TABLE 1

TITER OF IMMUNE SERUM COMPARED WITH NORMAL SERUM

	Serum dilution	Penicillinase added: mgm	Penicillinase added: units	Precipitins
Immune serum	Str.	0.25	100	++++
	1:2			++++
	1:4			++++
	1:8			+++
	1:16			++
	1:32			++
	1:64			+
	1:128			±
	1:256			0
	1:512			
Normal serum	Str.	0.25	100	0
	1:2			0
	1:4			0
	1:8			0
	1:16			0
	1:32			0
	1:64			0
	1:128			0
	1:256			0
	1:512			

It will be noted that a positive precipitin test was obtained in a serum dilution as high as 1:256, whereas the normal serum was negative in all dilutions. Experiments are now being conducted to further increase the antibody content of the immune sera. Further work will be initiated to obtain the gamma globulin fraction by purification of the serum, which presumably contains practically all the antibody activity.[6]

There are three main routes for the inactivation or excretion of penicillin[7] in the body, the gastro-intestinal tract, the renal system, and oxidation, reduction or conjugation.[8] The inactivation in the gastro-intestinal tract is caused to a considerable extent by the penicillinase produced by certain intestinal bacteria.

It was therefore believed that the combination of this immune serum globulin fraction with penicillin will not only delay the action of penicillin but should also protect it from inactivation by penicillinase producing organisms in the animal body. This work is now under investigation and will be reported at a later date.

SUMMARY

It has been found that an anti-penicillinase immune serum can be produced in rabbits by the use of purified penicillinase as the antigen.

Anti-penicillinase serum is suggested as protection for penicillin from inactivation by penicillinase.

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[2] E. B. McQuarrie, A. J. Liebmann, R. G. Kluener and A. T. Venosa, *Archiv. Biochem.*, 5: 307, 1944.

[3] Supplied by Mr. E. B. McQuarrie and associates of the Biochemical Division of Schenley Research Institute.

[4] J. T. Culbertson, *Jour. Immunol.*, 23: 439, 1932.

[5] J. G. Baier, *Proc. Soc. Exp. Biol. and Med.*, 27: 421, 1929.

[6] J. F. Enders, *Jour. Chem. Invest.*, 23: 510, 1944.

[7] K. H. Beyer, L. Peters, R. Woodward, W. F. Verwey, *Jour. Pharmacol. and Exp. Ther.*, 82: 310, 1944.

[8] D. Perlstein, H. E. Wright and A. J. Liebmann, SCIENCE, 101: 562, 1945.

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DAVID PERLSTEIN
ALFRED J. LIEBMANN
SCHENLEY RESEARCH INSTITUTE
LAWRENCEBURG, IND.

BLOOD LEVELS AND URINARY EXCRETION IN PEANUT OIL, BEESWAX AND PENICILLIN MIXTURE

CLINICALLY, penicillin has proven to be a highly effective drug, yet its application in non-hospitalized patients is a difficult problem because of its rapid utilization and excretion from the body. This has necessitated repeated injections at frequent intervals; a procedure that more or less disrupts both patients' and doctors' daily schedules. Recently several methods of prolonging the action of this drug have been described. Of these, the most promising is the use of a penicillin-peanut oil and four per cent. beeswax mixture, as described by Romansky and Rittman[1] and confirmed by Zinnamon and Seeberg.[2] The prolongation is achieved by delaying the absorption of penicillin from the area injected.

Recently, an opportunity to use this mixture was afforded this department, and the data obtained pertaining to the excretion of this drug form the basis of this report. The clinical results obtained were good, but will not be considered here, as a more complete report of these findings has been published by the U. S. Public Health Service.

TECHNIQUE

The material used was a mixture of peanut oil and four percent. white beeswax containing 100,000 units of calcium penicillin per cc.[3]

Urine and blood were assayed by the serial dilution "turbidimetric" method of M. H. Dawson and G. L. Hobby.[4] The number of Oxford units were determined by comparing bacterial inhibition of serial dilutions of the fluid to be tested and sets of penicillin solutions of known potency determined by assay against a reference standard of penicillin calcium obtained from the Food and Drug Administration of the Federal Security Agency. The bacteria used were the Oxford strain of hemolytic *Staphylococcus aureus*. This is not the method best suited for the determination of minute amounts of penicillin, as this strain is inhibited by 0.1 units per cc. In our hands, however, it is rapid, practical, and gives easily reproducible results.

The drug was administered intramuscularly in a single dose of 200,000 units. Blood and urine specimens were assayed at the intervals shown in the following table and interval and total excretion values were calculated. A total of 23 patients were treated. The values shown represent the average amounts of penicillin found for each interval. The minimum and maximum amounts are also given.

TABLE 1

PENICILLIN EXCRETION FROM BEESWAX AND PEANUT OIL MIXTURE

Time interval hrs.	Blood levels (Units per cc)			Urine levels (Units per cc)			Urine levels (Total units excreted)		
	Low	High	Average	Low	High	Average	Low	High	Average
2	.225	.45	.343	45.	135.	109.5	3,600	18,225	10,660
4	.225	.45	.235	33.7	135.	83.6	3,375	18,900	8,790
6	0	.23	.152	22.5	135.	75.3	0	17,100	7,672
8	0	.225	.020	22.5	90.	61.4	1,854	14,400	6,884
10				22.5	135.	69.1	2,812	11,250	5,838
12				22.5	135.	54.3	2,250	13,500	5,333
14				22.5	90.	48.4	1,800	13,500	5,323
16				0	67.5	38.1	0	14,087	4,495
18				0	90.	35.1	0	7,414	3,104
20				0	90.	33.4	0	6,000	2,495
22				0	67.5	23.5	0	4,275	2,233
24				0	67.5	20.0	0	5,063	1,786
24 to 36				0	45.	9.5	0	15,750	4,866
36 to 48				0	11.5	1.7	0	5,175	640
Totals . .							15,691	164,639	70,119

In short, our findings confirmed the prolongation of demonstrable blood levels as reported by Romansky and Zinnamon. An additional fact, however, which was noted and considered of more import than has previously been accorded it, was the even more prolonged presence and concentration of penicillin in the urine. The implications of this will be considered later.

DISCUSSION

It will be noted that penicillin could be detected in significant amounts in the blood for an average of over five hours. Since all reports[5, 6, 7] thus far agree that the water or saline solutions of penicillin are excreted in 3 to 4 hours, this is a definite prolonga-

[1] Romansky and Rittman, *Bull. U. S. Army Med. Dept.*, No. 81, p. 43, October, 1944.

[2] Zinnamon and Seeberg, *Venereal Disease Information*, 26: 2, 27, February, 1945.

[3] Prepared and furnished by Squibb in cooperation with the U. S. Public Health Service.

[4] Dawson and Hobby, personal communication.