the glucuronic acid content in urine in a typical animal experiment. The time of penicillin treatment is indicated. A twenty-five-day test period was used.

The glucuronic acid of the untreated animals was found to be within the normal range for rabbits, as set forth by Deichmann.[8] However, 24 hours after penicillin treatment, a marked increase in glucuronic acid was quantitatively demonstrable by the methods of both Deichmann[8] and Hanson.[9]

Analyses for solids, specific gravity, pH, sugar and albumin were also daily made, but since there was no significance in the data they are not recorded.

For the purpose of comparison, the excretion of penicillin as organic sulfates was also determined and the results recorded as per cent. of inorganic to total sulfates. The method of Treon and Crutchfield[10] was used for these determinations. Since there was no definite trend in the values obtained other than normal variation, it is concluded that penicillin does not influence the *in vivo* excretion of sulfates.

RESULTS

The normal 24-hour excretion of glucuronic acid in rabbit urine maintained on a diet of Purina rabbit chow, fresh carrots and cabbage ranges from 25 to 60-mg.

Following treatment of rabbits with penicillin sodium, a marked increase in glucuronic acid was noted. The treated animals gradually returned to normal.

Tests were made to determine whether or not penicillin sodium itself gives a color reaction characteristic of the naptha-resorcinol-glucuronic acid method of both Deichmann[8] and Hanson.[9] The results of the investigation in both instances were negative.

DISCUSSION

It is the general opinion of most investigators that approximately 60 per cent.[11] of penicillin administered can be recovered from the excreted animal urine. Since in this investigation it was found that there was an immediate sharp increase in the glucuronic acid content of rabbit urine following the intravenous administration of penicillin, it would appear likely that some part of the unaccounted-for 40 per cent. of penicillin normally excreted from the animal body conjugated with glucuronic acid.[4]

The data suggest the possibility of using the quantitative determination of glucuronic acid in urine to detect the presence and the extent of absorption of penicillin.

It is further possible that, since commercial penicillin contains certain impurities, the increased glucuronic acid in urine may be due to the impurities of penicillin rather than penicillin itself. The experiments will be extended with more highly purified penicillin.

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NON-ACID-FAST FORMS OF THE MYCOBACTERIUM OF HUMAN LEPROSY*

IN 1939, the writer had the opportunity of staining several smears of material obtained at the Branch Laboratory of the New York State Department of Health from the nasal septum of a Mexican who had been diagnosed as an early case of leprosy.[1] The smears were stained by the author's Triple Stain method[2] which reveals non-acid-fast forms of *Mycobacterium tuberculosis* not disclosed by the usual Ziehl-Neelsen technic. When this method is applied to material from tuberculous lesions or cultures, acid-fast tubercle bacilli stain red, non-acid-fast forms (rods, granules, and the recently demonstrated zoogleal forms)[3] stain blue, while other organisms, tissue cells, etc., form a light green background.

The two leprosy smears stained in this manner showed acid-fast rods, and a few non-acid-fast forms. As soon as the patient became aware of the diagnosis of his affliction, he made an escape from supervision, and no further material could be obtained.

Recently, however, Dr. Frank Combes, professor of dermatology at New York University and chief of the Dermatology Service at Bellevue Hospital, suggested that material from cases of leprosy might reveal interesting non-acid-fast forms if stained by the author's Triple Stain technic. He kindly arranged to have a number of unstained fixed smears of nasal

* This work was supported by a grant from the Lessing J. Rosenwald Foundation.

[1] Dr. Morton C. Kahn informs me that in 1929 he visited Mahaika Leprosarium in British Guiana after completing his studies on the development of the tubercle bacillus, as he felt that non-acid-fast forms might be concerned in the pathology of leprosy. The finding of so many non-acid-fast saprophytes in the nasal cavity caused the work to be discontinued, and there was insufficient time for skin biopsies.

[2] E. Alexander-Jackson, SCIENCE, 99: 307, 1944.

[3] E. Alexander-Jackson, *Annals of the New York Academy of Sciences* (in press).

[8] Wm. Deichmann, *Jour. Lab. and Clin. Med.*, 28: 770, 1943.

[9] S. W. Hanson, G. T. Funch Mills and R. T. Williams, *Biochem. Jour. England*, 38: 3, 274, 1944.

[10] Treon and Wm. Crutchfield, Jr., *Ind. Eng. Chem., Anal. Ed.*, 14: 119, 1942.

[11] C. K. Rammelkamp and C. S. Keefer, *Jour. Clin. Invest.*, 22: 425, 1943.

material sent to the writer from a case of leprosy at the Willard Parker Hospital. These smears were stained by the Triple Stain method and examined. They revealed not only red-staining acid-fast bacilli, but blue-staining non-acid-fast rods in even greater numbers. Some of the nests of rods were mixtures of the two types, but many nests consisted of blue-staining rods only.

Dr. Combes then secured from Dr. F. A. Johansen, Dr. G. H. Faget and Dr. G. L. Fite, of the U. S. Marine Hospital at Carville, La., a box of fixed unstained smears made from leprous skin biopsies. Each preparation represented a different case. These were all stained by the writer's Triple Stain method and carefully examined. The results are given in Tables 1 and 2.

TABLE I

THIRTY-TWO LEPROSY SMEARS STAINED BY THE TRIPLE STAIN TECHNIC*

Type lesion	Patient's number	Date of admission	Acid-fast		Non-acid-fast	
			Rods	Zoogleals and granules	Rods	Zoogleals and granules
Lepromatous	651	1929	0	0	+	+
	1530	1942	+	0	0	?
	1644	1944	0	+	+	+
Neural	118	1921	0	0	0	+
	653	1929	0	0	0	+
	1536	1942	0	0	0	+
Tuberculoid	1634	1944	0	0	0	+
	1679	1944	0	0	0	+
Maculo-Anesthetic	699	1930	+	0	+	+
	1651	1944	0	0	0	+
Mixed	859	1932	0	0	0	+
	884	1932	+	0	+	+
	941	1933	0	0	0	0
	1073	1935	0	?	0	?
	1150	1936	+	0	+	0
	1280	1938	+	0	+	+
	1332	1939	0	0	0	+
	1375	1940	0	0	0	+
	1377	1940	0	+	0	+
	1393	1940	0	0	0	+
	1509	1942	+	0	+	+
	1510	1942	0	0	0	+
	1511	1942	0	0	0	+
	1514	1942	0	0	0	+
	1552	1942	+	0	+	+
	1590	1943	0	0	0	+
	1604	1943	0	+	0	+
	1606	1943	+	+	+	+
	1617	1944	+	+	+	+
	1652	1944	0	0	0	+
Type not known	1354	1939	0	0	0	+
Nasal	Willard Parker	1944	+	+	0	0

* Only three smears showed presence of contaminating organisms.

Of the 32 cases, 27 smears (84.4 per cent.) showed zoogleal and granule or spore-like forms; 16 smears (50 per cent.) showed zoogleal forms only; 13 smears (40.6 per cent.) showed some acid-fast forms; 11 smears (34.4 per cent.) showed acid-fast or non-acid-fast rod forms. The findings are apparently unrelated to the dates of admission, as there was no greater tendency for smears from older cases to show greater numbers of rod forms. The stained preparation from the oldest case, admitted twenty-four years ago (1921), showed non-acid-fast zoogleal forms only.

It was interesting to note the presence of zoogleal forms and absence of rods in all the smears from the group of six neural and tuberculoid cases. Bacilli are usually lacking or difficult to demonstrate in these types of lesions.[4]

Occasionally, forms were found in which frankly acid-fast rods and granules lay within a blue-staining zoogleal mat. Some of the zoogleal forms were acid-fast, and some were semi-acid-fast.

TABLE II

ENUMERATION OF FINDINGS ON THIRTY SMEARS FROM SKIN BIOPSIES TAKEN FROM LEPERS AT CARVILLE, LA.

Totals	Type lesion	Rods	Zoogleal forms	Granules or spore-like bodies
3	Lepromatous	2	2	3
3	Neural	0	3	3
2	Tuberculoid	0	2	1
2	Maculo-Anaesthetic	1	2	2
20	Mixed	7	16	5
30		10	25	14

While relatively few cases are reported here, and *Mycobacterium leprae* can not as yet be cultured on suitable media or successfully inoculated into animals[5] in order to obtain absolute experimental proof, nevertheless these findings strongly suggest that the mycobacterium of human leprosy, like the mycobacterium of tuberculosis, has a zoogleal form or phase. The existence of non-acid-fast forms in leprosy may explain certain peculiarities in the course of the infection, such as its protracted incubation period, and the difficulty in demonstrating bacilli in certain types of lesions. The results of this limited study would seem to encourage more extensive observations with the aid of the Triple Stain technic.

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[5] M. H. Soule and E. B. McKinley, *ibid*, 87.