the amount of inositol present. However, the claim that this antagonism is of the competitive type was not rigorously established, since the antagonist : inositol ratio giving half-maximal growth was not determined over a wide range. In mice and rats the protective action of the normal metabolite is observed only when it is provided in larger amounts than the antagonist; also it appears that as the dose of methylene oxide is increased, protection is maintained only by an increase in ratio of inositol to antagonist. On this basis the antagonism between *myo* methylene oxide and *myo*-inositol in animals is of the non-competitive type as defined by Woolley(10), and is perhaps to be compared with the antagonism between Dicumarol® and vit. K.

That *myo* methylene oxide produces a necrotic lesion in the kidney cortex is perhaps not surprising, since such lesions are caused by many toxic agents. The necrosis is unusual, however, in that it remains narrowly confined to the juxtamedullary region under a wide variety of dosage regimens. The evidence suggests that kidney failure is the primary cause of death from doses of 1-1.5 times the MLD, but some contribution from other effects is indicated by the fact that survival time of the treated animals is usually slightly less than average survival time after bilateral nephrectomy(11,12).

A logical interpretation of the injurious effect of a *myo*-inositol antagonist on the kidney would be that *myo*-inositol has a particularly critical role in kidney metabolism. Such an inference is not yet warranted, however. *Myo* methylene oxide is a 1,2-epoxide, which places it in the class of monofunctional alkylating agents. It is not unlikely that this property, rather than its cyclitol nature, is primarily responsible for the toxic action of the compound. Presumably the fact that it is a cyclitol, differing only slightly from *myo*-inositol in configuration, is the basis for the antagonism of its action by the inositol.

Investigations of the effects of *myo* methylene oxide on *myo*-inositol metabolism will be reported elsewhere.

The authors are grateful to Prof. J. J. Lalich, Dept. of Pathology, for histological examinations; and to Miss Margaret Olson, Clinical Laboratory, School of Medicine, for urea nitrogen and creatinine determinations.

1. Hawthorne, J. N., *J. Lipid Research,* 1960, v1, 255.
2. Burns, J. J., *Am. J. Med.,* 1959, v26, 740.
3. Schopfer, W. H., Posternak, T., *Schweiz. Z. allgem. Pathol. u. Bakteriol.,* 1956, v19, 647.
4. Posternak, T., *Biochem. Preparations,* 1952, v2, 57.
5. ———, *Helv. Chim. Acta,* 1944, v27, 457.
6. Litchfield, J. T., Wilcoxon, F., *J. Pharmacol. Exp. Therap.,* 1949, v96, 99.
7. Skeggs, L. T., *Am. J. Clin. Path.,* 1957, v28, 311.
8. Peters, J. H., *J. Biol. Chem.,* 1942, v146, 179.
9. Angyal, S. J., Anderson, L., *Advances in Carbohydrate Chem.,* 1959, v14, 135.
10. Woolley, D. W., *A Study of Antimetabolites,* John Wiley and Sons, New York, 1952, p68.
11. Durlacher, S. H., Darrow, D. C., *Am. J. Physiol.,* 1942, v136, 577.
12. Mason, M. F., Casten, G., Lindsay, A., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 303.

Received October 17, 1962. P.S.E.B.M., 1963, v112.

Degradation of Insulin by Tissue Extracts of Normal and Nephrotic Rats.* (27982)

H. L. WILDBERGER, H. T. RICKETTS AND LIDIA REGUT
Department of Medicine, University of Chicago

Insulin requirements are decreased in some patients following onset of diabetic glomerulosclerosis(1,2,3). Analogously, induction of nephrotoxic serum nephrosis in rats with alloxan or pancreatectomy diabetes diminishes blood and urinary glucose(4,5,6). We(6) have shown that I^{131} insulin injected intravenously disappears from the blood more

* This work was supported by grant from Nat. Inst. of Arthritis & Metab. Dis., P.H.S.

TABLE I. Hypoglycemic Effect in Normal Rats of Insulin Incubated with Tissue Extracts.

Avg of 30 and 40 min blood	Insulin (10)	Insulin and kidney extract		Insulin and liver extract	
		Normal (20)	Nephrotic (13)	Normal (13)	Nephrotic (9)
Avg of 30 and 40 min blood sugars as per cent of initial value	51.5	70	61.5	81.5	61
		($p < .01$)		($p < .002$)	

Figures in parentheses indicate No. of animals.

slowly in nephrotic than in normal rats. In view of the marked affinity of the normal kidney for insulin(7) it seemed possible that these clinical and experimental observations might be explained by a decreased ability of the damaged kidney to sequester or inactivate insulin, either exogenous or endogenous, thus leaving more free circulating hormone for the metabolism of glucose. Studies with labeled insulin, however, failed to demonstrate any relation between its disappearance time in blood and its fixation by kidney, liver or muscle in normal, nephrotic and nephrectomized rats(6). Tissue extracts of such animals therefore were assayed for their ability to inactivate insulin.

Methods. (1) Ten rats (Holtzman, Sprague-Dawley) were made nephrotic by injection of rabbit nephrotoxic serum(8). By a modification of Mirsky's method for insulinase(9), kidneys, livers and diaphragms from these and from 8 normal rats were homogenized separately in a Waring blendor for 15 seconds in 4, 3 and 6 volumes, respectively, of cold normal saline. The homogenate was centrifuged at 1500 rpm under refrigeration for 20 minutes. The supernate was filtered and brought to pH 7.5 with N/10 NaOH. A mixture of this filtrate and commercial insulin (1 ml of filtrate to 18 units of insulin in 1 ml of buffer) was incubated for one hour at 37°C. The extract thus prepared from each organ was diluted 1 to 10 with commercial insulin buffer and 0.5 ml (containing 0.2 unit of insulin) injected I.V. into each of 10 to 14 lightly anesthetized (nembutal) normal rats weighing 250 to 300 g and fasted 4 hours. A similar group of normal rats was given 0.2 unit of insulin alone. Blood for glucose determination was taken before and at 30, 40 and 60 minutes after injection. The fall in blood sugar, using the average of the 30 and 40 minute values, was expressed as percentage of the initial fasting level.

(2) Portions of the tissue extracts described above were incubated for one hour at 37°C with .005 millicurie of I^{131} insulin per ml and diluted to 200 volumes with 25% human serum. Aliquots, representing total radioactivity, were counted in a well-type counter. After precipitation with an equal volume of 10% TCA, the suspension was centrifuged and filtered and the filtrate counted for radioactivity. Counts per minute (cpm) of the precipitate were calculated by difference. Preparations identical in content and treatment were heated to 80°C for 10 minutes. Radioactivity of samples ranged from 8000 to 15,000 cpm and background from 200 to 300 cpm.

Results and discussion. (1) Insulin plus extracts from nephrotic kidney and liver gave a significantly greater fall in blood sugar than insulin plus extracts from normal organs, approaching the effect of insulin alone (Table I). Thus, nephrosis lessened insulin inactivation by liver and kidney extracts. Extracts of "nephrotic" diaphragm, however, gave results similar to those obtained with normal diaphragm. The kidney and liver extracts which inactivated appreciable amounts of insulin represented relatively minute amounts of tissue (5 mg of kidney), indicating the considerable potential of the intact organs.

(2) Extracts of normal kidney, liver and diaphragm incubated with I^{131} insulin contained 43%, 39%, and 48%, respectively, of total radioactivity in TCA precipitate. Similar extracts from nephrotic rats contained 51%, 52% and 68% in the precipitate. All extracts heated at 80°C for 10 minutes showed 97% of total radioactivity in the precipitate. Since, under these conditions, the

radioactivity of the precipitate is thought to represent undegraded insulin(7), the conclusion is again justified that organ extracts (including in this case diaphragm) from nephrotic animals inactivate insulin less well than such extracts from normal animals.

It is suggested that the smaller amount or activity of a heat-labile insulin-degrading substance ("insulinase"?)(9) contained in the tissues of nephrotic as compared with normal rats may account for the observed(6) longer persistence in the circulation of I.V. injected I^{131} insulin in rats with renal disease.

Summary. 1. The insulin-degrading capacity of rat kidney, liver and diaphragm extracts was determined by incubating them with (a) crystalline insulin and observing blood sugar curves in intact rats following intravenous injection of the mixture and (b) I^{131} insulin and measuring undegraded insulin as represented by TCA precipitable radioactivity. 2. By both measurements, kidney and liver extracts from nephrotic rats were less potent than normal organ extracts in the inactivation of insulin.

1. Zubrod, C. G., Eversole, S. L., Dana, G. W., *New Eng. J. Med.*, 1951, v245, 518.

2. Runyan, J. W., Jr., Hurwitz, D., Robbins, S. L., *ibid.*, 1955, v252, 388.

3. Epstein, F. H., Zupa, V. J., *ibid.*, 1956, v254, 896.

4. Kalant, N., Clamen, N., Hoffman, M. M., *Diabetes,* 1958, v7, 140.

5. Creutzfeldt, W., Frericks, H., Malsch, D., Moench, A., *Arch. Exp. Path. u. Pharmak.,* 1959, v236, 392.

6. Ricketts, H. T., Wildberger, H. L., Regut, L., *Diabetes,* in press.

7. Elgee, N. J., Williams, R. H., Lee, N. D., *J. Clin. Invest.,* 1954, v33, 1252.

8. Heymann, W., Lund, H. Z., *Pediatrics,* 1951, v7, 691.

9. Mirsky, I. A., Broh-Kahn, R. H., *Arch. Biochem.,* 1949, v20, 1.

Received October 19, 1962. P.S.E.B.M., 1963, v112.

Distribution of Polyoma Virus Antibodies in Japan. (27983)

TATSUHEI KONDO AND HIDEHIRO MATSUURA (Introduced by G. E. Moore)
Department of Surgery, School of Medicine, Nagoya University, Nagoya, Japan

Polyoma virus has been isolated from leukemic mice. It has been found to infect exposed animals very readily, especially those housed in the same room with the leukemic mice(1). Some American mice have been imported into Japan, and there is a strong possibility that polyoma virus has been brought into Japan in them. We have surveyed 12 widely separated mouse colonies in Japan for the presence of polyoma virus antibodies.

Materials and methods. For the present study, mice imported from the United States or Germany, as well as some Japanese mice, were obtained from 12 mouse colonies in Japan (Table I). The study involved the imported strains A, AKR, cb, CBA, C_{57}BL, C_3H, C_3HxDD, DD, DM, Hr^{rh}, R_{III}, RF, Swiss/albino, Swiss/Hauschka, and Zb, and the Japanese strains D_{103} and DDO. On arrival at the laboratory, mice were bled by heart puncture. The serum was heated at 56° C for 30 minutes immediately before use in hemagglutination inhibition tests.

The antigen was polyoma virus strain 2609 from Dr. S. Stewart, and was grown in tissue culture. Pregnant Swiss mice were sacrificed with ether, and the entire embryos were removed aseptically, minced with scissors, and then subjected to trypsinization. After centrifugation, the cells were suspended in an LH medium containing 10% pooled calf serum and small amounts of penicillin and streptomycin. The resulting suspension was transferred to tissue culture bottles and incubated at 36°C. A few days later, when confluent cell sheets had developed, the cultures were inoculated with the virus. As soon as early cytopathogenic changes were observed, the fluids were collected and were assayed for hemagglutinin content with a standard pool of mouse serum. The HA titers of the virus used for this study were 1/254 or higher.