Whether medium 199 in which LH-v had been serially passed was concerned in its genetic instability cannot be answered at present.

Titration in TC of the spinal cords of inoculated mice indicated that LH-a probably multiplied in the host to a lesser degree than LH-v, although both variants multiplied equally well in MKTC. In this respect they behaved like the virulent and attenuated strains of poliovirus in monkeys.

Summary. Both mouse-avirulent LH-a and mouse-virulent LH-v variants of Leon (Type III) poliovirus represented mixed populations, but LH-a yielded more avirulent plaques while LH-v yielded more virulent plaques. On replating, virulent and avirulent plaques usually yielded corresponding virulent and avirulent progeny, although intermediate forms sometimes appeared. After purification, virus from 3 avirulent plaques derived from LH-v was serially passed in Eagle's medium and in 2 of them reversion to virulence occurred; however, in another set of experiments, virus from 2 avirulent plaques derived from LH-a was passed in the same way and reversion did not occur. Titration in TC of suspension of the spinal cords of some inoculated mice indicated that LH-v multiplied more readily in the host than LH-a.

1. Li, C. P., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 354.
2. Dulbecco, R., Vogt, M., *J. Exp. Med.*, 1954, v99, 167.
3. ———, *Ann. N. Y. Acad. Sci.*, 1955, v61, 790.
4. Hsiung, G. D., Melnick, J. L., *Immunol.*, 1957, v78, 128.
5. ———, *ibid.*, 1958, v80, 282.
6. Li, C. P., Jahnes, W. G., *Virology*, 1956, v2, 828.

Received March 27, 1958. P.S.E.B.M., 1958, v98.

Experimental Aminonucleoside Nephrosis.(I): Action of Cortisone on Aldosterone and Corticosterone Production. (24035)

D. DAS GUPTA AND C. J. P. GIROUD (Introduced by J. S. L. Browne)
Endocrine Research Laboratory, Montreal Children's Hospital and Department of Investigative Medicine, McGill University, Montreal, Canada

Singer(1) has shown that the nephrotic-like syndrome, induced in rats by an aminonucleoside related to puromycin(2,3) is accompanied by an increased rate of production of aldosterone as measured in the adrenal vein effluent. The purpose of the present work was to confirm this finding and to study the rate of secretion of aldosterone and corticosterone by the adrenals of normal and nephrotic immature rats treated with cortisone acetate.

Methods. Male hooded rats with starting body weight of 75 to 85 g were used. Nephrosis was induced by single daily subcutaneous injections of 6-dimethylaminopurine, 3-amino-d-ribose (1.75 mg in 0.2 ml of water/rat/day for 8 days). Cortisone acetate was given in single daily intramuscular injections for 5 consecutive days, beginning, in nephrotic animals, 24 hours after the last dose of the nephrotoxic drug. Unless otherwise specified, all animals were bled 16-18 hours after being last injected with either the nephrotoxic drug, or cortisone. Adrenal vein blood was collected according to operative procedure of Bush(4) under nembutal anesthesia (7.5 mg/100 g of rat). Since the rate of aldosterone and corticosterone secretion tends to fall markedly with time after one hour of bleeding (personal communication), the period of blood collection was limited to one hour. Blood from 2 to 5 rats was pooled and extracted as described by Bush(4). Residues were submitted to a single paper chromatographic separation in the benzene-aqueous methanol system(5), at room temperature (25 ± 2°C) 5 hours, after overnight equilibration. Corticosterone was eluted with methanol in a modified Haines' device(6), and quantitatively estimated, in duplicate samples, at 2400 Å according to the method of Saffran and Schalley(7). Spectrophotometric meas-

TABLE I. Aldosterone and Corticosterone in Adrenal Vein Blood of Normal Untreated Rats.

No. rats in pool	Mean wt, g	Mean vol blood collected, ml/adr/kg/hr	μg/kg/adrenal/hr Aldosterone (as DOCA equiv.)	μg/kg/adrenal/hr Corticosterone
3	90	24	33	94
3	93	24	43	105
3	84	24	20	92
4	93	23	38	91
5	90	22	23	80
4	101	24	25	89
Mean ± S.E.			30 ± 3.8	92 ± 8.5

urements of this steroid were performed in methanol solution against a paper blank. A standard curve using cortisone was established with each set of determinations. Corticosterone was furthermore characterized by soda fluorescence, reduction of blue tetrazolium, mixed chromatography and sulfuric acid chromogen. In the chromatographic system used, aldosterone is found in the section of the chromatogram extending from bottom of hydrocortisone to that of cortisone, as defined by mobility of these hormones used as pilots. In none of our experiments was aldosterone or any other Δ^4-3-ketosteroid present in this section in amounts sufficient to be detected under ultra-violet light (mineral light $\lambda = 2540$). This portion of each chromatogram was cut into small pieces which were soaked and shaken twice during 30 minutes with 20 ml of methanol. This eluate was brought to dryness and eventually bioassayed by the Simpson-Tait method(8). Its biological activity is hereafter referred to as aldosterone. In all instances the aldosterone content of normal and nephrotic rat adrenal vein blood was bioassayed and, at the same time that of the respective groups of animals treated with cortisone. In each assay the sodium retaining response of test animals was assessed by using 3 standard doses of desoxycorticosterone acetate. The level of aldosterone in rat adrenal vein blood is therefore expressed as desoxycorticosterone acetate equivalent (DOCA equiv.). Under the conditions of our experiment, preliminary results indicated that in this assay crystalline d-l aldosterone 21-monoacetate is more than 100 times as potent as desoxycorticosterone acetate. Following injections of the nephrotoxic drug, the development of experimental nephrotic syndrome was established on the basis of decreased serum albumin, changes in electrophoretic pattern of plasma proteins, hypercholesterolemia and ascites. In nephrotic animals, changes in electrophoretic pattern of plasma proteins were grossly characterized by marked decrease of albumin and elevation of α-1 globulin fractions. The magnitude of hypoalbuminemia and hypercholesterolemia was, in general, comparable to that reported by previous investigators(3).

Results. Secretion of aldosterone and corticosterone in adrenal vein blood of normal untreated rats is equal to 30 ± 3.8 μg DOCA equiv./kg/adrenal/hr and 92 ± 8.5 μg/kg/adrenal/hr respectively (Table I).

In the nephrotic animals, one series of rats was studied 16 to 18 hours and the other series 6 days after the last of 8 injections of aminonucleoside. The results are presented in Table II. Sixteen to 18 hours after last injection of the drug the nephrotic animals show a definite increase of aldosterone secretion as compared to that in normal animals (157 ± 9.3 μg DOCA equiv/kg/adr/hr: P ± .001), and a less striking but also significant increase of corticosterone secretion (143 ± 18.2 μg/kg/adr/hr: P<.01). The fact that these changes in adrenal secretion persist despite discontinuance of injections is proven by the finding of a comparable aldosterone (181 ± 29.3 μg DOCA equiv/kg/adr/hr) and corticosterone (172 ± 15.5 μg/kg/adr/hr) increase 6 days after withdrawal of the drug. In certain groups this is accompanied by a further increase of fluid retention as judged by greater accumulation of ascites (Table III).

When normal rats received cortisone at dose level of 100 to 500 μg intramuscularly/day for 5 days, the expected decrease of corticosterone secretion was noted (Table IV). Examination of mean values of corticosterone produced by animals receiving 350 and 500 μg of cortisone/day shows that injection of this hormone reduced the secretion of corticosterone to about ⅓ of values found in controls (Table I). Under cortisone administration the secretion of aldosterone was higher

TABLE II. Aldosterone and Corticosterone in Adrenal Vein Blood of Nephrotic Rats ("Early" Nephrotic).*

No. of rats in pool	Mean wt, g	Mean ascitic fluid, ml/kg body wt	Mean vol blood collected, ml/adr/kg/hr	μg/kg/adrenal/hr	
				Aldosterone (as DOCA equiv.)	Corticosterone
4	100	38	21	75	140
3	96	43	20	120	148
3	111	41	23	261	207
4	99	42	25	227	83
3	112	34	16	104	107
3	93	35	30	156	174
Mean ± S.E.		39 ± 1.5		157 ± 9.3	143 ± 18.2

* Nephrotic Syndrome induced by 6-aminonucleoside of puromycin: 1.75 mg/100 g body wt inj. subcut. daily 8 days. Animals were bled on ninth day: 16 to 18 hr after last inj. of drug.
Compared to that of normal rats the secretion of aldosterone by the nephrotic animals is very significantly increased: P = .001.

than the controls in all groups. Since the magnitude of the increase varies markedly with dose of cortisone and within different groups receiving the same dose, it is only of moderate statistical significance (P = .02). It may however be of interest to emphasize that, in spite of this increased aldosterone secretion which in 4 instances reached levels found in nephrotic animals, no signs of fluid retention were detected in these rats.

The action of cortisone on adrenal secretion of nephrotic rats is presented in Table V. At dose level of 350 and 500 μg/day, cortisone caused in 4 groups out of 7 a decrease of aldosterone secretion to normal values, whereas in the 3 remaining groups the hormone was depressed below normal in 2 groups and remained slightly above normal in one. Considered together, the values of aldosterone in the nephrotic series treated with 350 and 500 μg of cortisone are not significantly different from those of normal untreated animals (P< .1). Cortisone treatment of nephrotic animals, especially at the higher doses, resulted in a decrease of corticosterone secretion comparable to that observed in normal animals treated with identical amounts of cortisone (Table IV).

In the nephrotic series, administration of cortisone was accompanied by a marked decrease, or by disappearance of ascites. The values for ascitic fluid volume of nephrotic rats treated with 350 and 500 μg of cortisone/day are highly significantly different from those of untreated series measured either 16-18 hours or 6 days after last injection of the nephrotoxic drug (P<.001). Decrease in aldosterone secretion and in volume of ascites was not accompanied by changes in electrophoretic pattern of plasma proteins.

Discussion. The present results, although obtained with a different strain of animals,

TABLE III. Aldosterone and Corticosterone in Adrenal Vein Blood of Nephrotic Rats ("Late" Nephrotic).*

No. of rats in pool	Mean wt, g	Mean ascitic fluid, ml/kg body wt	Mean vol blood collected, ml/adr/kg/hr	μg/kg/adrenal/hr	
				Aldosterone (as DOCA equiv.)	Corticosterone
3	130	43	25	161	125
3	97	52	20	197	138
4	102	95	18	319	182
3	113	27	23	137	180
3	133	61	17	150	233
3	112	30	20	125	176
Mean ± S.E.		51 ± 10.2		181 ± 29.3	172 ± 15.5

* Animals were bled 6 days after last inj. of puromycin.

TABLE IV. Aldosterone and Corticosterone in Adrenal Vein Blood of Normal Rats Treated with Cortisone Acetate.*

Cortisone, μg/100 g body wt/day	No. rats in pool	Mean wt, g	Mean vol blood collected, ml/adr/kg/hr	μg/kg/adrenal/hr: Aldosterone (as DOCA equiv.)	μg/kg/adrenal/hr: Corticosterone	
100	4	94	23	60	70	
200	3	83	30	67	79	
350	3	84	27	130	37	
"	4	92	18	—	28	32
"	4	100	25	280	31	
500	2	95	20	126	27	
"	4	97	19	74	16	
"	3	95	25	45	9	17
"	4	87	23	75	13	
"	3	102	21	129	20	

* Cortisone acetate, Merck (lot #K.E. 207). Single daily intramusc. inj. for 5 days as aqueous microcrystalline suspension.

Animals were bled 16 to 18 hr after last inj. of cortisone. Compared to those of normal untreated controls the values of aldosterone of all cortisone treated groups are significantly elevated: $P = .02$.

fully confirm the finding of Singer(1) who showed that rats rendered nephrotic by 6-dimethylaminopurine, 3-amino-d-ribose present an increased rate of secretion of aldosterone. According to our results a less marked but appreciable increase of corticosterone secretion seems to be a rather constant feature of this experimental syndrome. However, the values of mean ratio aldosterone-corticosterone of 2 untreated nephrotic series (1.09 and 1.04) when compared to that of the normal animal (.34) emphasize that development of nephrosis is accompanied by a much higher rate of production of aldosterone than of corticosterone. One may point out that the observed changes in rate of secretion of aldosterone and corticosterone cannot be explained on the basis of a difference of blood flow through the adrenals, since in practically all our groups the mean volume of adrenal vein blood collected is quite comparable when expressed/adrenal/kg of body weight/hr.

The increased aldosterone secretion by normal rats treated with cortisone is an unexpected finding. Cortisone possesses some sodium retaining activity in the Simpson-Tait

TABLE V. Aldosterone and Corticosterone in Adrenal Vein Blood of Nephrotic Rats Treated with Cortisone Acetate.*

Cortisone, μg/100 g body wt/day	No. rats in pool	Mean wt, g	Mean ascitic fluid, ml/kg body wt	Mean vol blood collected, ml/adr/kg/hr	μg/kg/adrenal/hr: Aldosterone (as DOCA equiv.)	μg/kg/adrenal/hr: Corticosterone	
200	3	103	25	19	171	125	
350	3	115	24	17	27	68	
"	3	110	traces	27	39	46	69
"	3	98	"	24	7	93	
500	3	110	25	29	4	20	
"	4	109	15	23	24	40	28
"	3	100	16	20	53	32	
"	3	105	traces	21	22	21	

* Cortisone acetate, Merck (lot #K.E. 207) single intramusc. inj. daily for 5 days beginning 24 hr after last inj. of puromycin. Animals were bled 16 to 18 hr after last inj. of cortisone acetate.

Aldosterone values in nephrotic rats treated with 350 and 500 μg of cortisone are not significantly different from those of normal untreated controls.

Mean and stand. error of ascitic fluid vol of groups treated with 350 and 500 μg of cortisone: 12.3 ± 3.0.

bioassay(8). One could therefore expect that a certain amount of injected hormone is present in the general circulation and, assuming that it remains unchanged by passing through the adrenal, is therefore present in the collected adrenal vein blood effluent. If this was the case the chromatographic zone eluted for biological assay of aldosterone would contain cortisone (see methods). However, if one were to attribute to cortisone the sodium retaining activity observed, it would be necessary to assume the presence of this hormone on the chromatograms, in amounts readily detectable under ultra-violet light. It has already been pointed out that on none of the chromatograms did the aldosterone zone contain detectable amounts of Δ^4-3-ketosteroid material. It is therefore concluded that injection of cortisone in normal rats has led to an appreciable increase of aldosterone secretion. To a limited extent these findings are comparable to those of Farrell *et al.*(9) who showed that after long-term cortisone treatment the aldosterone secretion of a group of 4 normal dogs was increased moderately in 2 cases and appreciably in a third. However the overall aldosterone secretion in treated dogs, when compared to that of normal controls, proved to be statistically insignifiacnt. It is possible to assume that injection of cortisone in normal rats might in some way alter the electrolyte balance of animals, possibly by producing sodium diuresis, which, secondarily would be responsible for an increased output of aldosterone. At the present time this remains purely hypothetical.

Treatment of nephrotic animals with cortisone resulted in a decrease of rate of production of aldosterone and marked diminution of ascites. Since in certain groups the volume of ascitic fluid in untreated nephrotic rats increases sharply in the 6 days of observation following the last injection of the nephrotoxic drug (Tables III and IV), the action of cortisone in the nephrotic series may be interpreted both as decreasing ascites and preventing its further accumulation. These results are at variance with those of Fiegelson *et al.* (3) who were unable to demonstrate increased rate of production of aldosterone by the adrenals of aminonucleoside nephrotic rats, and to influence the degree of ascites by cortisone treatment. These discrepancies might in part be explained by the fact that these authors assessed the production of aldosterone of their animals by incubation of the adrenal glands, and that the dose of cortisone used by them was some 5 to 7 times higher than the effective doses used in the present study.

The present data do not provide information on the primary mode of action of cortisone in nephrotic rats in the sense that they do not disclose whether the decrease of aldosterone production is due to a direct action of cortisone at the adrenal level, or to its systemic action on the disease process, with secondary readjustment of mineralo-corticoid function. It is hoped that the results of further investigations will help to clarify this problem.

Conclusion. An increased rate of aldosterone production after induction of aminonucleoside nephrosis in rats has been confirmed. After short term treatment with cortisone acetate, at the dose level of 350 and 500 μg/day, corticosterone production of both normal and nephrotic animals was decreased to one-third of their respective controls. Cortisone reverted the increased aldosterone secretion of nephrotic rats to normal, this was accompanied by marked loss or even total disappearance of ascites.

The authors wish to express their deep gratitude to Dr. B. Singer who laid the foundation of this work in Montreal Children's Hospital, to Dr. S. Hardy from Lederle for a generous gift of 6-dimethylaminopurine, 3-amino-d-ribose; to Dr. F. Gross and Dr. C. W. Murphy, CIBA Co. for aldosterone; to Dr. J. H. Laurie, Merck Co. for cortisone acetate; to Dr. Frances McCall and Dr. Eleanor Harpur for friendly interest and help during this study. We are specially indebted to Miss A. Henry for the electrophoretic studies. This work has been supported by grants from Nat. Research Council of Canada and the Research Fund of Montreal Children's Hospital.

1. Singer, B., *Endocrinology,* 1957, v60, 420.

2. Frank, S., Antonowicz, J. M., Craig, J. M., Metcoff, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 424.

3. Fiegelson, E. B., Drake, J. W., Recant, L., *J. Lab. and Clin. Med.,* 1957, v50, 437.

4. Bush, I. E., *J. Endocrin.*, 1953, v9, 95.

5. ———, *Biochem. J.*, 1952, v50, 370.

6. Nowaczynski, W., Koiw, E., Genest, J., *Can. J. Biochem. Physiol.*, 1957, v35, 425.

7. Saffran, M., Schalley, A. V., *Endocrinology*, 1955, v56, 523.

8. Simpson, S. A., Tait, J. F., *ibid.*, 1952, v50, 150.

9. Farrell, G. L., Banks, R. C., Koletsky, S., *ibid.*, 1956, v85, 104.

Received March 31, 1958. P.S.E.B.M., 1958, v98.

Detection of Minute Gastrointestinal Bleeding Utilizing Radioactive Iron, Fe^{59}.* (24036)

EARL G. YONEHIRO,† HARLAN D. ROOT,‡ JOHN F. PERRY, JR.,§ JAMES F. MARVIN,‖ AND OWEN H. WANGENSTEEN

Departments of Surgery and Radiology, University of Minnesota Medical School, Minneapolis, Minn.

A simple method for detecting minute gastrointestinal bleeding would provide a screening test for detection of ulcerated lesions of the alimentary tract not recognizable by conventional radiographic or other currently available diagnostic means. Such a test could be utilized for detection of the presence of silent cancers of mucous membranes of the gastrointestinal canal, or possibly of other tracts with external orifices. The use of radioactive ferrous citrate, Fe^{59}, for this purpose was investigated. Radioactive iron given orally may make its appearance in red blood corpuscles as early as 4 hours following ingestion(1). Radioactive iron given intravenously is also rapidly incorporated into the red blood cell(2). Bleeding from the mucous membrane of the alimentary tract should be recognizable by the appearance of increased radioactivity in the stool provided normal excretion patterns of the isotope by this route are determined.

The experiments reported herein indicate that occult bleeding in the gastro-intestinal tract can be detected by the use of radioactive ferrous citrate incorporated into the red blood cell. After preliminary study, the method appears to be more sensitive than the guaiac test for this purpose.

Method. Twenty healthy mongrel dogs weighing 10-15 kg, and divided into 3 groups, were utilized in this study. Group I consisted of 8 control animals, each of which received 25 microcuries of radioactive ferrous citrate (Fe^{59}) intravenously. Group II consisted of 6 test animals. Each of these received 25 microcuries of radioactive ferrous citrate intravenously and after 14 days also received 30 mg of histamine in beeswax intramuscularly daily. Ulcerations of the gastrointestinal tract occur regularly in the majority of animals so treated(3). Group III consisted of 6 normal dogs. Each of these received by gastric tube small amounts of Fe^{59} tagged blood obtained from one of the Group I animals. In all animals of Groups I and II following injection of the isotope and in Group III dogs following intragastric injection of the labeled blood, daily stool collections were made. An equal amount of water was added to each 24-hour stool sample and mixed thoroughly in Waring Blendor. Four ml of resultant mixture was then counted in a well-type scintillation counter, 1000 or more counts being recorded for each specimen. Values are expressed as number of counts/4 ml of stool-water mixture for 10 minutes, which actually represents the number of counts/2 ml aliquot of stool. Whole blood was removed by venipuncture from animals of Groups I and II at intervals, and one ml was counted in the scintillation counter. The guaiac test for occult blood was

* Supported by Institutional Grant of Am. Cancer Soc. and Malignant Disease Research Fund, Univ. of Minn.

† Fellow of Am. Can. Soc.

‡ Trainee, Nat. Cancer Inst., U.S.P.H.S.

§ Special Trainee, U.S.P.H.S.

‖ Department of Radiology.