

resuspension prior to nitrogen determination. The results of these experiments are shown in Table IV. A very close correlation between

TABLE IV. Relationship between Nitrogen Content and Virus Particle Counts of Washed Suspensions.

Virus suspension No.	Particles/ml ($\times 10^6$)	$\mu\text{g N/ml}$	Particles/ $\mu\text{g N}$ ($\times 10^7$)
136-1	4.6	47	9.8
-3	6.0	68	8.8
137-1	10.0	97	10.3
-2	10.0	102	9.8
-3	9.1	96	9.5

virus counts and nitrogen content is evident, with the average being 9.7×10^7 particles per μg of nitrogen.

Discussion. These results have demonstrated that the virus particle counts of meningopneumonitis virus in allantoic fluid obtained by this technic are consistently parallel to egg infectivity titers and nitrogen content. In 9 separate determinations number of particles per egg LD_{50} ranged from 83 to 240 with an average of 145. In 5 separate determinations number of particles per μg of nitrogen ranged from 8.8×10^7 to 10.3×10^7 .

It is certain that numerous non-elementary body forms have been included in the counts done by this technic but the proportion of these to elementary bodies is seldom more than 1:1 as seen by electron micrographs of virus in allantoic fluid and usually the large

forms account for about $\frac{1}{4}$ of the countable particles in electron micrographs. For rapid estimation of virus content of allantoic fluids this technic has been of particular value, and allows the selection of high titer fluids as well as use of a narrow range of dilutions for egg LD_{50} determinations.

The use of latex particles for this procedure has several advantages. This material is stable on storage for long periods of time, is readily available in a variety of particle sizes and is easily standardized by counting in a bacteria counting chamber. The particles are easily visualized by phase contrast microscopy and are readily differentiated from the virus.

Summary. A rapid method for estimating the meningopneumonitis virus content of allantoic fluid suspension is described. Evidence is presented which indicates that counts obtained closely parallel egg infectivity titers and nitrogen content determinations. In 9 separate experiments one egg LD_{50} was found to contain 83-240 virus particles as counted by this method.

1. Gogolak, F. M., *J. Infect. Dis.*, 1953, v92, 248.
2. Backus, R. C., Williams, R. C., *J. Appl. Physics*, 1950, v21, 11.
3. Crocker, T. T., *J. Immunol.*, 1954, v73, 1.
4. Reed, L. J., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
5. Lanni, F., Dillon, M. L., Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1950, v74, 4.

Received December 29, 1958. P.S.E.B.M., 1959, v100.

Hepatic and Splenic Uptake of Colloidal Radiogold in Rat after Partial Hepatectomy.* (24692)

KURT STERN AND ARLENE DUWELIUS

Mount Sinai Medical Research Fcn., and Depts. of Pathology, Mount Sinai Hospital and Chicago Medical School, Chicago, Ill.

The subject of this study, *viz.*, phagocytic activity of hepatic and splenic macrophages in the rat after partial hepatectomy, has received only scant attention.

* Supported by grant from Illinois Division, Am. Cancer Soc., and Ann Langer Cancer Research Fcn., Chicago, Ill.

Materials and methods. Healthy $2\frac{1}{2}$ to 6 months old Wistar and Lewis rats were used. Twenty-four hours before hepatectomy or sham operation, they were injected intraperitoneally with 0.2 to 0.3 $\mu\text{g/g}$ body weight of radiogold (Abbott's Aurcoloid). Removal of approximately $\frac{2}{3}$ of liver was done according

TABLE I. Hepatic Uptake of Au¹⁹⁸ in Lewis Rats after Hepatectomy.

Group	Sex	No. of rats	% of inj. Au ¹⁹⁸ per g of liver, mean $\pm$ S.D.	P
A Sham	♂	25	1.79 $\pm$.74	<.001
Hepatectomy	"	28	3.30 $\pm$ 1.22	
Sham	♀	16	4.26 $\pm$ 1.96	>.3
Hepatectomy	"	22	4.86 $\pm$ 2.54	
B Sham	♂	17	1.98 $\pm$.61	<.001
Hepatectomy	"	21	3.40 $\pm$ 1.14	
Sham	♀	16	3.59 $\pm$ 1.49	<.05
Hepatectomy	"	13	4.92 $\pm$ 1.38	
C Sham	♂	11	1.39 $\pm$.68	>.5
Hepatectomy	"	15	1.26 $\pm$.50	
Sham	♀	12	2.88 $\pm$ 1.42	>.5
Hepatectomy	"	16	2.72 $\pm$ 1.08	
Liver removed at hepatectomy	♂	65	1.26 $\pm$.99	<.001
	♀	52	3.34 $\pm$ 1.82	

A, 3 days after operation; B, 8 days after operation; C, 16 days after operation.

to methods described by Higgins and Anderson(1) and Brues *et al.*(2). Sham operation consisted of laparotomy equivalent to that performed for hepatectomized animals and of brief evulsion of the 2 anterior liver lobes from the incision. Liver samples from hepatectomized animals were weighed, homogenized with distilled water and formamide according to the method by Lahr *et al.*(3) and assayed for radioactivity by liquid counting in Geiger-Mueller counter. A second injection of radiogold of similar dosage was administered 24 hours before anticipated time of sacrifice, which took place 3, 8, 15 or 16 days, respectively, after hepatectomy. Immediately after death of animals, livers and spleens were completely removed, weighed, and converted into tissue homogenates for radioassay. Radioactivity was calculated for whole liver and spleen as well as per g of liver and spleen, with due consideration given to radiodecay of Au¹⁹⁸, and expressed as % of injected radiogold. When calculating radioactivity in liver of hepatectomized animals, radioactivity found in liver removed at time of hepatectomy was subtracted from activity of first injection of Au¹⁹⁸ given to the animal. Mean values and their standard deviations were determined according to usual methods. Tests for statistical significance of differences between values

were carried out according to student's *t* test.

Results. Table I summarizes data on uptake of radiogold/g of liver of Lewis rats. Sham-operated and hepatectomized rats of same sex are compared with each other. Three days after hepatectomy, male animals showed significantly greater uptake in the liver than did sham-operated ones. In female rats, there was only a slight difference in favor of hepatectomized animals 3 days after operation. Eight days following hepatectomy or sham operation, higher uptake in male hepatectomized rats was highly significant, and in female rats of borderline significance. Sixteen days after hepatectomy, male and female rats showed values essentially identical with those found in controls. In all instances, females showed significantly higher hepatic uptakes than did males of comparable groups.

Table II deals with uptake of radiocolloid in spleens of these rats. Three days after operation, in male hepatectomized rats the uptake was about twice as great as in controls whereas in female animals no such difference was found. Eight days after operation, male and female hepatectomized rats showed an increase over controls of borderline significance. Sixteen days after operation, there was no essential difference between hepatectomized and control males, and in female hepatectomized rats the uptake was lower than

TABLE II. Splenic Uptake of Au¹⁹⁸ in Lewis Rats after Hepatectomy.

Group	Sex	No. of rats	% of inj. Au ¹⁹⁸ per g of spleen, mean $\pm$ S.D.	P
A Sham	♂	25	1.89 $\pm$ 1.36	<.001
Hepatectomy	"	28	3.90 $\pm$ 1.72	
Sham	♀	15	4.01 $\pm$ 1.70	>.5
Hepatectomy	"	22	4.22 $\pm$ 1.97	
B Sham	♂	17	2.23 $\pm$ 1.25	<.1
Hepatectomy	"	21	2.92 $\pm$ 1.02	
Sham	♀	15	2.74 $\pm$ 1.30	<.1
Hepatectomy	"	13	3.70 $\pm$ 1.60	
C Sham	♂	11	2.82 $\pm$ 1.69	>.1
Hepatectomy	"	15	2.00 $\pm$.87	
Sham	♀	12	4.06 $\pm$ 2.34	>.1
Hepatectomy	"	15	2.91 $\pm$ 1.40	

A, 3 days after operation; B, 8 days after operation; C, 16 days after operation.

TABLE III. Hepatic Uptake of Au¹⁹⁸ in Male Wistar Rats after Hepatectomy.

Group	No. of rats	% of inj. Au ¹⁹⁸ per g of liver, mean $\pm$ S.D.	P
A Sham	24	1.51 $\pm$.84	<.001
Hepatectomy	23	2.92 $\pm$ 1.44	
B Sham	17	1.71 $\pm$.76	>.3
Hepatectomy	18	1.97 $\pm$.79	
Liver removed at hepatectomy	41	1.04 $\pm$.71	

A, 3 days after operation; B, 15 days after operation.

in controls, though the difference was not statistically significant. In the spleen, too, uptake of Au¹⁹⁸ was greater in females than in males of corresponding groups.

In male Wistar rats phagocytic activity was estimated 3 and 15 days, respectively, after hepatectomy. As shown in Table III, uptake of radiogold in liver was markedly increased 3 days after hepatectomy, whereas 15 days later there was no significant difference. Table IV shows Au¹⁹⁸ uptake in spleen of hepatectomized Wistar rats was significantly increased 3 days and 15 days after hepatectomy.

Since uptake of Au¹⁹⁸ was also known for hepatectomy specimens these values were compared with those obtained at time of sacrifice. In Table V, the last vertical column refers to number of rats in which at sacrifice Au¹⁹⁸ uptake/g of liver was at least twice as great as in the hepatectomy specimen. Two-thirds of male Lewis rats showed ratios over 2, three days after hepatectomy, and three-fourths of them 8 days after hepatectomy, but only 1 of 15 rats, 16 days after hepatectomy. About 1/2 or 1/3 respectively of female Lewis

TABLE IV. Splenic Uptake of Au¹⁹⁸ in Male Wistar Rats after Hepatectomy.

Group	No. of rats	% of inj. Au ¹⁹⁸ per g of spleen, mean $\pm$ S.D.	P
A Sham	24	1.77 $\pm$.99	<.001
Hepatectomy	22	2.44 $\pm$.83	
B Sham	17	1.55 $\pm$.86	<.01
Hepatectomy	18	1.95 $\pm$ 1.01	

A, 3 days after operation; B, 15 days after operation.

rats showed increased uptake 3 and 8 days after hepatectomy, and none 16 days after hepatectomy. Of male Wistar rats, roughly 2/3 showed increased uptake 3 days after hepatectomy, and 1/2 at 15 days.

An additional effect of partial hepatectomy was noted in weights of spleens. Comparison of splenic weights expressed as % of body weight disclosed in male and female hepatectomized Lewis rats 8 days after hepatectomy a significant increase in relative size of spleen as compared with controls, and an increase in hepatectomized Wistar rats, 3 and 15 days after hepatectomy of borderline significance.

TABLE V. Comparison of Au¹⁹⁸ Hepatic Uptake Determined at Hepatectomy and at Sacrifice.

Strain	Sex	Days after hepatectomy	Total	No. of rats $\frac{S}{H} > 2^*$
Lewis	♂	3	28	18
	♀	3	22	10
	♂	8	20	16
	♀	8	13	4
	♂	16	15	1
	♀	16	16	0
Wistar	♂	3	23	16
	♂	15	18	10

$$* \frac{\text{Uptake at sacrifice}}{\text{Uptake at hepatectomy}} > 2.$$

Discussion. Our data suggest that after partial hepatectomy of rats, liver and spleen are endowed with increased phagocytic activity. This may be the result of proliferation of hepatic and splenic macrophages, an assumption supported by increase observed in relative splenic size and preliminary histologic studies. While female rats showed greater phagocytic activity than males, the effect of hepatectomy was less pronounced in female than in male rats, possibly because it is more difficult to stimulate phagocytosis operating near maximum efficiency.

Benacerraf and co-workers(4) observed that partial hepatectomy and other measures reducing portal blood flow in rats, interfered with blood clearance of intravenously injected india ink, but enhanced hepatic phagocytosis, presumably because of increased blood flow through remaining portion of liver. It is conceivable that increased blood flow/volume of

tissue following partial hepatectomy may have contributed to the increase of phagocytosis also in our experiments, but since we measured phagocytosis 24 hours after intraperitoneal injection, it is less likely that this was the decisive factor. Simultaneously with preliminary report of our results(5), Leong and associates(6) presented data obtained by isolated rat liver preparations perfused with radioactive colloidal chromic phosphate. In this study the authors found that 48 hours after hepatectomy phagocytic activity of liver had doubled over that of controls. Since these results were obtained with controlled rates of perfusion *in vitro*, they do not support the assumption that increased rate of blood flow was the main factor in elevating hepatic phagocytosis after partial hepatectomy. Abercrombie and Harkness(7) observed that regeneration of littoral cells proceeded at a faster rate than that of parenchymal cells between 2 and 7 days after partial hepatectomy. Similarly, Aterman(8) noted an increase in sinusoidal cells as early as 3 hours after partial hepatectomy. It is also of interest to refer to observations by Haven *et al.*(9) on increased immune responses in rats after partial hepatectomy; when injections of sheep red cells were given immediately after hepatectomy, these animals showed significantly higher hemolysin titers than did controls.

Summary. 1. Uptake of Au¹⁹⁸ in liver and spleen was compared after hepatectomy or

sham operation was carried out in Lewis and Wistar rats. 2. Female rats showed higher uptake of radiogold in liver and spleen than did males. 3. Three and 8 days after hepatectomy, male Lewis rats, and 3 days after hepatectomy male Wistar rats, showed greater uptake of radiogold in liver and spleen than did sham-operated controls. Similar but less pronounced differences were found in female Lewis rats. 4. Fifteen or 16 days after hepatectomy male and female Lewis rats showed no significant differences in hepatic or splenic uptake of radiocolloid, while in male Wistar rats splenic but not hepatic uptake was still increased. 5. Increased spleen/body weight ratios were observed in hepatectomized rats.

1. Higgins, G. M., Anderson, R. M., *Arch. Path.*, 1931, v12, 186.
2. Brues, A. M., Drury, D. R., Brues, M. C., *ibid.*, 1936, v22, 658.
3. Lahr, T. N., Olsen, R., Gleason, G. I., Tabern, D. L., *J. Lab. and Clin. Med.*, 1955, v45, 66.
4. Benacerraf, B., Biozzi, G., Cuendert, A., Halpern, B. N., *J. Physiol.*, 1955, v128, 1.
5. Stern, K., Duwelius, A., *Fed. Proc.*, 1958, v17, 458.
6. Leong, G. F., Pessotti, R. L., Brauer, R. W., *ibid.*, 1958, v17, 388.
7. Abercrombie, M., Harkness, R. D., *Proc. Roy. Soc. London s. B.*, 1951, v138, 544.
8. Aterman, K., *Arch. Path.*, 1952, v53, 197.
9. Havens, W. P., Jr., Schlosser, M. E., Klatchko, J., *J. Immunol.*, 1956, v76, 46.

Received December 29, 1958. P.S.E.B.M., 1959, v100.

The Free Myo-Inositol of Thyroid Tissue.* (24693)

NORBERT FREINKEL, REX M. C. DAWSON, SIDNEY H. INGBAR AND ROBERT W. WHITE
*Thorndike Memorial Lab., Second and Fourth Medical Services (Harvard), Boston City Hospital,
 and Dept. of Medicine, Harvard Medical School, Howard Hughes Medical Inst.,
 and Biochemistry Dept., A. R. C. Inst. of Animal Physiology,
 Babraham, Cambridge, England*

During a study of phospholipid metabolism of sheep thyroid tissue(1-3), abundant quantities of a water-soluble compound were noted which exhibited chromatographic mobility and

staining characteristics of free myo-inositol. In addition, in lipid extracts of sheep thyroid tissue, an inositol-containing phosphoglyceride was demonstrated. *In vitro*(2), as well as *in vivo*(3), the P³² turnover in this phosphoinositide was 2 to 10 times more rapid

* The research was supported in part by U.S.P.H.S. grants and Surgeon General's contract.