Biochemical Pharmacology, 1962, Vol. 11, pp. 1137–1140. Pergamon Press Ltd., Printed in Great Britain.

ENLARGEMENT OF THE LIVER AND SPLEEN IN MICE OF THE O_{20} (AMSTERDAM) STRAIN CAUSED BY STORAGE OF MACROMOLECULAR SUBSTANCES—I

THE STORAGE OF THE MACROMOLECULAR SUBSTANCES DEXTRAN AND POLYVINYLPYRROLIDONE IN THE LIVER AND SPLEEN

A. E. F. H. MEIJER

Laboratory for Pathology, Department of Biochemistry, University of Leiden, Holland

(*Received* 1 *June* 1962; *accepted* 20 *August* 1962)

Abstract—Mice of the O_{20} (Amsterdam) strain were intraperitoneally injected with the macromolecular substances dextran and polyvinylpyrrolidone. Quantitative determinations were made of the amounts of these substances stored in the liver and spleen. It appeared that only a very limited amount of the injected quantity of the macromolecular substances was stored. The injections caused enlargement of the liver and spleen in the mice.

IN PREVIOUS investigations it was observed that when mice of the O_{20} (Amsterdam) strain were injected intraperitoneally with solutions of macromolecular substances a part of these injected substances was stored in the liver and spleen.[1] Simultaneously, the liver and spleen increased in weight: The liver by about 20 per cent of the original weight, and the spleen by about 200 per cent. In these studies use was made of various polymers of vinylpyrrolidone (PVP) with an average molecular weight varying between 17,000 and 640,000, dextran with an average molecular weight of 160,000, and a carbon suspension with an average particle diameter of 250 Å. In contradistinction to the metabolically inert polymers of vinylpyrrolidone[2, 3] and the metabolically inert carbon particles, the dextran[4, 5] is broken down slowly in the animal organism.

Investigation with light microscopy demonstrated that the macromolecular substances are stored in the cytoplasm of both the parenchymal and Kupffer cells. In the spleen the macromolecular substances are stored only in the cytoplasm of the reticulum cells.[6]

Electron microscope observations are in complete agreement with these findings:[7–9] they demonstrate that in both organs dextran and PVP are stored in organellae in the cytoplasm of parenchymal cells and of cells belonging to the histiocytic system.

In connexion with our studies, in which changes in the enzyme activity in the liver and spleen resulting from the storage of macromolecular substances were investigated, it was of interest to determine whether the increase in weight in both organs was caused by the weight of the stored quantities of macromolecular substances. Moreover, as the used macromolecular substances can inhibit the activity of the enzyme β-glucuronidase,[10] it was for our investigations necessary to investigate the quantities of the substances storaged in the liver and spleen. In this work, the dextran and PVP contents were quantitatively determined for the liver and spleen. The results are given below.

MATERIALS AND METHODS

Mice of the O_{20} (Amsterdam) strain, from 6 to 8 months old, were used. After intraperitoneal injection of dextran and PVP, the animals were killed by dislocation of the cervical vertebrae. Dextran and PVP were quantitatively determined in homogenates of the liver and spleen.

Macromolecular substances

1. Dextran, (average molecular weight 160,000) from Poviet & Co., Amsterdam.
2. Polyvinylpyrrolidone, a polymer of vinylpyrrolidone (average molecular weight 640,000) from Bayer, Leverkusen.
3. Polyvinylpyrrolidone (average molecular weight 12,600) from Bayer, Leverkusen.

All three substances were used as a sterile 6% solution in 0·9% NaCl.

Injection scheme

Five groups of animals were used. Group I was given a daily intraperitoneal injection of 1 ml of dextran solution for 9 days and fasted for 18 hr before being killed on the tenth day. Group II was given a daily intraperitoneal injection of 1 ml of dextran solution for 3 days and fasted for 18 hr before being killed on the fourth day. Group III was given a daily intraperitoneal injection of 1 ml of dextran solution for 3 days, received no treatment for 4 days, and fasted for 18 hr before being killed on the eighth day. Group IV was given a daily intraperitoneal injection of 1 ml of polyvinylpyrrolidone solution (12,600) for 9 days and fasted 18 hr before being killed on the tenth day. Group V was given a daily intraperitoneal injection of 1 ml of polyvinylpyrrolidone solution (640,000) for 9 days and fasted 18 hr before being killed on the tenth day.

Determination of dextran

Dextran was determined according to Turner and Maycock,[11] but in order to exclude any adsorption of dextran on the filter paper, centrifugation was used throughout to remove precipitates from the solutions. In addition, orcinol was used instead of anthrone[12] for the colour reaction.

Determination of polyvinylpyrrilodone

Polyvinylpyrrolidone was determined according to a modification of Zipf's method.[13]

RESULTS AND DISCUSSION

In each liver the dextran or the PVP content was determined. The sensitivity of the methods used for the determinations was such that the quantity of the incorporated substances in the spleen was too small to permit determination of the content per organ, and therefore the average quantity per group was determined in the spleen.

The data in Table 1 indicate that only a very small part of the injected quantity of the macromolecular substances is stored in the liver and spleen. Appreciably more was stored in the liver than in the spleen. Expressed per gramme of tissue, however, this storage is practically the same for both organs. The mice which received three injections of dextran stored less in both liver and spleen than those which received nine injections of dextran. For the liver this difference was significant ($P < 0{\cdot}05$).

Because there were no individual observations, it is impossible to say whether the difference observed for the spleen is significant.

Although in contrast to dextran, polyvinylpyrrolidone is metabolically inert, the order of magnitude of the stored amounts is the same for the mice of Groups I and V.

TABLE 1. THE QUANTITIES OF DEXTRAN AND PVP STORED IN THE LIVER AND SPLEEN AND THE QUANTITIES OF DEXTRAN AND PVP STORED PER GRAMME WET TISSUE FOR THE FIVE DIFFERENT GROUPS OF MICE

Group	Treatment	Number of		Mg dextran or PVP in organ with s.d.‡		Mg dextran or PVP/g, wet tissue with s.d.‡	
		Mice	Injections†	Liver	Spleen	Liver	Spleen
I	dextran, 160,000*	9	9	12·0 ± 2·3	1·07	10·1 ± 2·6	11·0
II	dextran, 160,000*	9	3	8·2 ± 1·7	0·95	8·0 ± 2·4	10·4
III	dextran, 160,000*	9	3	7·4 ± 1·9	0·78	7·1 ± 1·4	8·2
IV	PVP, 12,600*	8	9	2·6 ± 0·9	0·12	2·5 ± 0·7	2·5
V	PVP, 640,000*	4	9	9·3 ± 1·2	1·34	8·3 ± 0·7	10·2

* Mol. weight.
† 60 mg. per injection.
‡ s.d. $= \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$

TABLE 2. THE WEIGHTS OF THE MICE AND OF THE LIVERS AND SPLEENS WITH THE STANDARD DEVIATIONS, FOR THE FIVE GROUPS OF MICE, AND THE WEIGHTS OF A CONTROL GROUP WHICH FASTED FOR 18 HR BEFORE THEY WERE INVESTIGATED

Group	Treatment	Average body weight* (g) with s.d.	Average liver weight (mg) with s.d.	Average spleen weight (mg) with s.d.
I	dextran 9×	20 ± 3	1189 ± 106	98 ± 19
II	dextran 3×	19 ± 2	1026 ± 96	91 ± 18
III	dextran 3×	20 ± 2	1048 ± 124	95 ± 25
IV	PVP 12,600 9×	18 ± 3	1053 ± 143	48 ± 8
V	PVP 640,000 9×	19 ± 3	1125 ± 156	132 ± 25
controls		20 ± 2	854 ± 72	37 ± 7

* Weight at the end of the experiment.

The elimination of the macromolecular substances dextran and PVP takes place via the kidneys. The rate at which the substances diffuse into the urine is, however, dependent on the molecular size and is more or less inversely proportional to the molecular weight of the injected macromolecules.[14–17] As a result, the elimination of the injected substances by the mice of Group IV during the injection period was

quantitatively greater than for the other groups. This is in all probability the reason why Group IV stored significantly less in the liver and spleen. It is also striking that the splenomegaly in this group was less obvious than that of the other groups (Table 2). This phenomenon has also been seen in other studies.[1]

Turner and Maycock[11] injected mice intravenously with solutions of dextran and determined the dextran content in various organs including the liver and spleen. The order of magnitude of the amount stored found by them is in agreement with our results.

Table 2 gives the weights of the animals and of both organs for all five groups of mice, as well as the corresponding data for a control group. This control group comprised seven mice, which fasted for 18 hr before examination. The data in Table 2 give an impression of the order of magnitude of the weight increases obtained with the present injection scheme. The weight increases resulting from the storage varied for the liver from about 200 to 300 mg and for the spleen from about 10 to 95 mg. It is in any case clear that the observed amounts of the macromolecular substances which were stored in both organs cannot be responsible for the observed increases in weight.

Acknowledgement—The author wishes to acknowledge the valuable technical assistance of Miss H. Williams Guy.

REFERENCES

1. A. E. F. H. MEIJER and R. G. J. WILLIGHAGEN, *Biochem. Pharmacol.* **8**, 389 (1961).
2. R. STEELE, D. D. VAN SLYKE and J. PLAZIN, *Ann. New York Acad. Sci.* **55**, 479 (1952).
3. H. A. RAVIN, A. M. SELIGMAN and J. FINE, *J. Med.* **247**, 921 (1952).
4. I. GRAY, *Amer. J. Physiol.* **174**, 462 (1953).
5. R. TERRY, C. L. YAILE, A. GOLODETZ, C. E. PHILLIPS and R. R. WHITE, *J. Lab. Clin. Med.* **42**, 6 (1953).
6. R. G. J. WILLIGHAGEN. Thesis, Leiden (1960).
7. J. C. H. DE MAN, W. TH. DAEMS, R. G. J. WILLIGHAGEN and TH. G. V. RIJSSEL, *J. Ultrastruc. Res.* **4**, 43 (1960).
8. G. GABLER, *Virchows Arch.* **333**, 230 (1960).
9. G. HÜBNER, *Beitr. pathol. anat.* **126**, 1 (1962).
10. A. E. F. H. MEIJER, *Biochem. Pharmacol.* **11**, 1153 (1962).
11. P. A. TURNER and W. D'A. MAYCOCK, *J. Path. Bact.* **75**, 145 (1958).
12. R. BOURRILLON, *C. R. Soc. Biol., Paris* **1**, 240 (1953).
13. A. E. F. H. MEIJER, *Clin. Chim. Acta* **6**, 713 (1961).
14. G. GROTTE, R. C. KNUTSON and J. L. BOLLMAN, *J. Lab. Clin. Med.* **38**, 577 (1951).
15. G. GIEBISCH, H. D. LAUSON and R. F. PITTS, *Amer. J. Physiol.* **178**, 168 (1954).
16. A. GRÖNWALL, H. HINT, B. INGELMAN, G. WALLENIUS and O. WILANDER, *Scand. J. Clin. Lab. Invest.* **4**, 363 (1952).
17. W. SCHOLTAN, *Z. ges. exp. Med.* **130**, 556 (1959).