Summary. Propionin injected intraperitoneally in mice has an L.D. 50 of 1.7 ml per kg. Propionin administered by stomach tube to rats has an L.D. 50 of 15.3 ml per kg.

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Non-Accumulation of Pectin Intravenously Injected into Rabbits.

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(Introduced by C. A. G. Wiersma.)

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A number of materials have been used as substitutes for blood in the treatment of shock and hemorrhage. Acacia solutions were used rather extensively for this purpose during World War I, mainly as a result of the work of Bayliss.[1] Later investigators have shown that there are many dangers attendant upon the intravenous use of acacia. Andersch and Gibson[2] reported that about one-half of the injected acacia accumulated in the liver. Yuile and Knutti[3] found that after repeated injections of acacia in dogs the liver weight increased 5 or 6 times and that sometimes as much as 8 to 10% of acacia (by weight) was found in the liver.

Pectin sols, having physical properties similar to those of acacia, should show the same advantages for transfusion purposes and yet, due to their different chemical constitution, might not cause damage by storage in the liver. The characteristic constituent of acacia, a gum exudate from certain tropical trees, is aldobionic acid which is unusually resistant to hydrolysis. Pectin, however, is a normal constituent of fruits and vegetables used for human food and is rather easily hydrolyzed in mildly acidic or alkaline systems and by many enzymes. Its molecule, with a weight of 150,000 to 300,000 is composed of chains of partially esterified galacturonic anhydride units.[4]

A general study of the effect of pectin in the circulatory system of rabbits has been in progress in this Department for the past 3 years. Various types of pectin in solutions of different concentra-

1 Bayliss, W. M., *Proc. Roy. Soc. (London) B.*, 1916, **89**, 380.
2 Andersch, M., and Gibson, R. B., *J. Pharm. Exp. Therap.*, 1934, **52**, 390.
3 Yuile, C. L., and Knutti, N. E., *J. Exp. Med.*, 1939, **70**, 605.
4 Joseph, G. H., *Bull. Natl. Formulary Comm.*, 1940, **9**, 18.

tions have been injected intravenously into rabbits, particular attention being given to the effects upon organs involved with the circulatory mechanism. No ill effects of any kind have been observed. Chemical analyses have shown that there is no deposition of pectin in the liver and kidneys of rabbits receiving large amounts of pectin intravenously over a period of several weeks.

Hartman, Schelling, Harkins, and Brush[5] of the Henry Ford Hospital in Detroit have recently proposed the use of pectin solutions as a blood substitute in the treatment of shock. Their preliminary experimental results as to the storage of pectin in the liver coincide with our findings. In view of the current interest in possible blood substitutes we submit some data from typical chemical analyses of liver, kidney and blood to supplement the work of Hartman and his associates.

Since the purpose, in our animal experiments, was to get excessive amounts of pectin into the blood, 6.0% pectin sols in normal saline were used. This is no doubt a greater concentration than would be desirable for transfusion purposes. Sols were made by dissolving 60 g of citrus pectin, which complied with the tentative standards of the forthcoming N.F. VII[6] in one liter of 0.85% sodium chloride solution. These sols were heated to 250°F for one hour, allowed to stand overnight to aid clarification, after which they were filtered into sterile containers. No attempt was made to adjust the pH of these sols to the region normal for blood. Although no discomfort has been noted as a result of administering pectin sols at their normal pH (3.2-3.8) some may wish to adjust the sols to higher pH values.

If pH adjustment of pectin sols is desired, it should be done after autoclave treatment because the molecular weight of pectin decreases rapidly at high temperatures when the pH is changed far from its natural region. Autoclaving the sols at their natural pH is usually just sufficient to bring the molecular weight down to the average molecular weight of human plasma proteins. Intravenous administration of unautoclaved pectin sols is probably not advisable because, due to the large size of the original pectin molecule, retention in body tissues might result.

The sols prepared as described above were injected in 10 ml amounts into the marginal ear vein, every other day, over the period from May 5 to July 7, 1941. The total volumes used were equivalent to approximately 15.0 g of dry pectin per rabbit. This amount of

[5] Hartman, F. W., Schelling, V., Harkins, H. N., and Brush, B., *Ann. Surg.*, 1941, **114**, 212.

[6] Powers, J. L., and Beeler, E. C., *Bull. Natl. Formulary Comm.*, 1940, **9**, 31.

pectin for a 4 kg rabbit would be equivalent to about 25 liters of a 1.0% solution for a 70 kg human, an amount far in excess of any normal use.

After completion of the 7 weeks' treatment the animals were autopsied. No abnormalities were found in the liver, spleen, kidneys, heart, lungs, or digestive tract. Analytical data on 4 rabbits so treated, together with 2 controls, are given in Table I. The method for determination of pectin in animal organs which we found reproducible and most satisfactory was developed by one of us (E.F.B.) from Youngburg's[7] method. The procedure is given below.

At autopsy the blood or organ is weighed and immediately frozen with dry ice. Before analysis, the organ is partially thawed, sliced paper thin with a sharp knife, and ground to a fine slurry in a mortar. Twenty-five grams of the slurry are extracted 3 times, each with 10 ml 20% trichloracetic acid for 5 minutes. The extracts are filtered into a 250 ml volumetric flask. The third acid extraction is followed by 2 hot water extractions. The residue on the filter is then washed with hot water until the flask is filled to the mark. After cooling to 20°C it is made to volume. Using 2.0 ml of extract (equivalent to 0.2 g of organ) and 5 ml of 85% phosphoric acid, the furfural distillation is made according to Youngburg. A small piece of paraffin in the distillation flask prevents foaming. Blanks on the organs of untreated animals should be deducted from the results. These blanks are similar to those found by Andersch and Gibson.[2]

The pectin used in our work formed 187.0 mg of furfural per gram, and since the method reveals as little as a few hundredths of a milligram of furfural, small amounts deposited in the organs could be detected.

As can be seen from the data in Table I, the furfural values for

TABLE I.
Furfural Recovery from Rabbit Organs After Repeated Pectin Sol Injections.

Rabbit		Total inj. (equivalent g dry pectin)	Mg furfural found per g of			Remarks
No.	Wt, kg		Liver	Kidney	Blood	
28	4.2	None	4.60	0.26	0.06	Control—No Pectin
29	3.4	None	3.68	*	*	" " "
20	4.2	15.0	4.30	0.26	*	Autopsied 3 days after last inj.
5	4.2	14.2	4.87	0.30	0.06	" 7 " " " "
27	4.2	14.1	6.38	*	*	" 3 " " " "
23	4.1	13.9	4.18	0.32	0.05	" 7 " " " "

*Not determined.

[7] Youngburg, G. E., *J. Biol. Chem.*, 1927, **73**, 599.

the animals receiving pectin are not very different from those of the control animals. If pectin had been stored in the liver to the extent found by Andersch and Gibson[2] for acacia, the furfural values for the livers of the pectin-treated animals would have been of the order of 15 to 18 mg. No attempt was made to determine the time during which the pectin remained in the blood. An analysis was made 7 days after the last injection merely to show that the pectin had been eliminated from the blood.

Our work so far indicates that: (1) Massive amounts of pectin in the form of autoclaved isotonic solutions may be injected into the blood stream of rabbits over a period of some weeks without causing any noticeable effect upon the internal organs of the animals. (2) Chemical analyses showed no deposition of pectin in the liver and kidneys. (3) No pectin could be found in the blood 7 days after injection.

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Utilization of Parenterally Administered Casein Digest for Synthesis of Proteins.*

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Intravenous or subcutaneous administration of casein digest (representing amino acids and polypeptides) as a means of parenteral nitrogen administration has been studied recently by Whipple and associates[1] in dogs previously depleted of plasma protein and on a low protein diet. Elman and Weiner[2] first reported that casein digests could be safely administered to man when certain precautions were observed. Clinical use of casein digests by parenteral administration in nephrotic children has been reported by Farr and associates,[3, 4] Shohl, Butler, Blackfan and MacLachlin,[5] and Shohl and

* This work was facilitated by a grant from Mead Johnson and Company, Evansville, Indiana.

1 Madden, S., Zeldis, L., Hengerer, A., Miller, L., and Whipple, G., *Science*, 1941, **93**, 330.

2 Elman, R., and Weiner, D., *J. A. M. A.*, 1939, **112**, 796.

3 Farr, L., *J. Ped.*, 1940, **16**, 679.

4 Farr, L., and MacFadyen, D., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 444.

5 Shohl, A., Butler, A., Blackfan, K., and MacLachlin, E., *J. Ped.*, 1939, **15**, 469.