

obtains a phospholipid-free extract of cholesterol and triglyceride (1, 2). Cholesterol may then be quantitatively determined by any one of the standard procedures, while triglycerides are conveniently estimated by a determination of the glycerol after saponification (3). Similar experiments on rat plasma showed that the extraction of cholesterol or triglyceride was not quantitative unless the particle size of the zeolite was reduced to 80 to 100 mesh.

*Methods.* Extraction of lipids was carried out as described by Van Handel and Zilversmit (3), except that the extraction was continued overnight. Stock Zeolite<sup>1</sup> was divided into 40 to 79 mesh and 80 to 99 mesh sizes. Silicic acid column chromatography was applied to a purified chloroform extract as described by Van Handel and Zilversmit (3).

Cholesterol was analyzed after KOH saponification and extraction with petroleum ether. In addition, 1 ml. of plasma was analyzed by direct saponification, as in the method of Abell *et al.* (4).

The cholesterol content of petroleum ether extracts was determined by the method of Zak *et al.* (5). Triglycerides of chloroform extracts were measured by the method of Van Handel and Zilversmit (3). Complete removal of phospholipids was evidenced in all extracts by phosphorus analysis according to Bartlett (6). The results are shown in Table 1. A better extraction of cholesterol and of triglyceride was noted when the particle size of zeolite was decreased to 80 to 100 mesh. Differences like the one described here were not found in human, rabbit, or dog plasma. It would seem that at least two differences could explain these observations: (a) the cholesterol and triglycerides in rat plasma are chemically different from that found in other animals or (b) the cholesterol and triglycerides in rat plasma are more strongly bound to protein. The

<sup>1</sup>Stock Zeolite-Taylor, obtained from W. A. Taylor Company, Baltimore, Md.

### Determination of cholesterol and triglycerides in rat plasma\*

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► By shaking human, dog, or rabbit plasma with chloroform in the presence of a synthetic zeolite, one

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TABLE 1

Experiment	1		2		3		4	
	Cholesterol	Triglyceride	Cholesterol	Triglyceride	Cholesterol	Triglyceride	Cholesterol	Triglyceride
Method	mg./cc.	mg./cc.	mg./cc.	mg./cc.	mg./cc.	mg./cc.	mg./cc.	mg./cc.
Zeolite								
Stock	0.331	0.359	0.416	0.609	0.517	0.928	0.449	0.780
40-79 Mesh	0.451	0.485	0.465	0.718	0.562	0.966	0.512	0.780
80-100 Mesh	0.605	0.584	0.589	0.843	0.697	1.011	0.618	0.861
Silicic Acid Column	0.559	0.589	0.617	0.811	0.710	1.049	0.674	0.884
Abell	0.580	—	0.619	—	0.709	—	0.690	—

latter explanation seems the more reasonable, since coarse zeolite, when added to a chloroform extract of rat plasma, did not appear to adsorb cholesterol or triglyceride.

REFERENCES

1. Forbes, J. C. *J. Lab. Clin. Med.* **16**: 520, 1931.
2. Forbes, J. C., and H. Irving. *J. Lab. Clin. Med.* **16**: 909, 1931.
3. Van Handel, E., and D. B. Zilversmit. *J. Lab. Clin. Med.* **50**: 152, 1957.
4. Abell, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall. *J. Biol. Chem.* **195**: 357, 1952.
5. Zak, B., N. Moss, A. J. Boyle, and A. Zlatkis. *Anal. Chem.* **26**: 776, 1954.
6. Bartlett, G. R. *J. Biol. Chem.* **234**: 466, 1959.

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