cases, the values were approximately a third less and in one case less than a half.

The relationship, if any, of the incidence of pneumonia in the adults to lead poisoning is unknown.

In view of Hindle's[4] report, an attempt was made to transmit the inclusions to animals fed standard diets. Kidney suspensions were prepared in broth-saline and injected subcutaneously into rats, mice and guinea pigs. Two experiments were performed using freshly harvested kidneys known to contain inclusion bodies. In one case blind passage was performed to the third generation. Ten rats, fourteen mice and two guinea pigs were injected. Inclusions were found in none.

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ANTAGONISM BETWEEN HEPARIN AND PLASMA TRYPSIN

HEPARIN was found to inhibit the digestive action of crystalline trypsin upon casein[1, 2] and the lack of inhibitory action upon commercial trypsin[3] was ascribed by Horwitt[4] to the probable presence of indefinite amounts of chymotrypsin in those preparations to be found in the market, since chymotrypsin is not inhibited by heparin. On the other hand, heparin is being largely used as therapeutic agent in thrombosis and recently[5] it was reported the protective action of heparin against necrosis produced by extreme local cold (frost bite). Since activation of plasma trypsin might constitute a common mediator in many manifestations following thrombosis and platelet disintegration, we have found it advisable to study the effect of heparin upon the proteolytic enzyme found in normal plasma. Trypsin is present in plasma in a free (I) and a bound (II) condition[6,7] and can be estimated following precipitation either with acetone (I) or with a 2.5 per cent. solution of trichloracetic acid (II), incubation of the whole precipitate (resuspended in buffer pH 8.4) for 48 hours and final estimation of the N P N. Heparin added either before precipitation or after the preparation was set up for incubation, had a strong inhibitory effect as shown in Table 1.

TABLE 1

Exp. No.	Material used	Amount of heparin* added	Trypsin (mgm NPN/100 ml plasma) Total	Free
I	(a) dogs plasma	0	91.4	31.9
	(b) same + heparin	2 mgs	85.6	3.5
	(c) same + heparin	5 mgs	34.8	−1.0
II	(a) dogs plasma	0	114.0	19.4
	(b) same + heparin	10 mgs	12.6	4.8
	(c) same + heparin	10 mgs	49.1	...

* The heparin used in those experiments was a crystalline-sodium salf of beef heparin (11 units per mgm) kindly supplied by Dr. L. B. Jaques of Toronto, Canada.
Note: In experiment II c, heparin was added after precipitation by trichloracetic acid and immediately before incubation.

The fact that heparin displays a definite inhibitory effect upon plasma trypsin when added before activation of the enzyme by addition of trichloracetic acid might be explained by assuming that it strengthens the effect of the natural inhibitor present in plasma. This agrees with Ferguson's view[7] that the polypeptide-like inhibitor of trypsin present in plasma might have acidic groups analogous to those of heparin or that heparin might constitute a prosthetic group for this inhibitor. A more extensive report will follow this note.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF A PLANIMETER IN VOLUME STUDIES OF EARLY EMBRYOS

THE shape of developing ova after the first cleavage division does not allow accurate volume calculation on the basis of diameter or radius, a fact which must at least partly explain the absence of data pertaining to this problem.

The volumes of more than eighty ova and blastocysts have been successfully ascertained by the planimetry of serial sections of known thickness.

Serial ten micron sections of the specimens were projected at two hundred diameters of magnification and the outline of each section was accurately traced on suitable paper. The average of ten planimeter readings was taken for each section and the values

[4] E. Hindle, *Nature*, 129: 796, 1932. E. Hindle and F. Coutelen, *Compt. Rend. Soc. Biol.* 111: 870, 1932.
[1] M. K. Horwitt, SCIENCE, 92: 89, 1940.
[2] A. J. Glazco and J. H. Ferguson, *Proc. Soc. Exp. Biol. and Med.*, 45: 43, 1940.
[3] J. A. Wells, C. A. Dragstedt, J. A. Cooper and H. C. Morris, *Proc. Soc. Exp. Biol. and Med.*, 58: 57, 1945.
[4] K. Lange, L. J. Boyd and L. Loewe, SCIENCE, 102: 151, 1945.
[5] A. Schmitz, *Z. physiol. Chem.*, 250: 37, 1937.
[6] N. K. Iyengar, K. B. Sehra and B. Mukerji, *Ind. med. Gaz.*, 57: 348, 1942.
[7] J. H. Ferguson, SCIENCE, 97: 319, 1943.

for each section of a specimen added together. The planimeter was adjusted to read in cm². Volume of the specimen was then calculated in the following manner:

$$\text{Volume} = \text{total area (in cm}^2) \times \frac{\text{thickness of section} \times \text{microns}^2 \text{ per cm}^2}{\text{diameters of magnification}^2}$$

$$\text{Volume} = \text{total area (in cm}^2) \times \frac{10 \text{ microns} \times 100{,}000{,}000 \text{ microns}^2}{200 \times 200}$$

$$\text{Volume} = \text{total area (in cm}^2) \times \frac{1{,}000{,}000{,}000 \text{ microns}^3}{40{,}000}$$

$$\text{Volume} = \text{total area (in cm}^2) \times 25{,}000 \text{ microns}^3$$

It has been found that figures for the volume of a specimen can be closely checked by repetition of the whole process including the tracing and planimetry.

It must be remembered that the two surfaces of a section of a spherical structure will have different areas unless the section is equatorial, but that in planimetry the section must be considered one of a cylinder. It may be possible to decrease such error by averaging the area of the two surfaces in order to approach the area of the mid-plane of the section. Whether such a technique would give more accurate absolute volume can not be stated, but it does not appear that it would offer advantages in determining relative volumes of different specimens. The same problem arises in connection with wax plate reconstruction where the wax plates must be cut perpendicular to the surface, thereby leaving edges which must be rounded off to produce a smooth contour.

The technique of planimetry is well known to the engineers but, so far as can be determined, this is its first direct application to volume studies of early embryos where it offers an easy and convenient method of determining the volume of irregular objects. Data resulting from this study will be presented in detail elsewhere.

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THE USE OF CHARCOAL TREATED PEPTONE IN MICROBIOLOGICAL ASSAYS

MICROBIOLOGICAL assays using the *Lactobacillus arabinosus* for the determination of nicotinic acid, biotin and pantothenic acid are widely employed.[1, 2, 3] Charcoal-treated casein hydrolysate forms the chief source of nitrogen in the medium used for these determinations. The preparation of the hydrolysate is laborious. If purchased commercially, it is expensive.

[1] E. E. Snell and L. D. Wright, *Jour. Biol. Chem.*, 139: 675, 1941.

[2] W. A. Krehl, F. M. Strong and C. A. Elvehjem, *Ind. Eng. Chem., Analyt. Ed.*, 15: 471, 1943.

[3] H. R. Skeggs and L. D. Wright, *Jour. Biol. Chem.*, 156: 21, 1944.

It has been found possible to replace the hydrolysate satisfactorily in media used for assay of biotin, niacin or pantothenate with charcoal-treated peptone. Blanks and maximal acid production obtained with the peptone are satisfactory and the preparation of the peptone is simple.

METHOD

100 grams of Bacto-Difco peptone are dissolved in 800 cc of distilled water. The pH of the solution is adjusted to 3.0 with concentrated HCl. A faint cloud forms at this point. Twenty grams of the activated charcoal, Darco G60, are added and the mixture stirred mechanically for an hour. The solution is then filtered by suction. The pH is readjusted to 3.0 with concentrated HCl, 10 grams of Darco G60 added, and the mixture stirred for an hour, after which it is filtered by suction. The filtrate should have no more than a faint tinge of color. The volume is adjusted to one liter and the solution preserved under toluene. Ten cc of this solution are substituted for each 5 cc of casein hydrolysate in the medium of Krehl, Strong and Elvehjem.[2] The peptone should be tested before being used for assays. If the peptone has been properly prepared, less than 2.0 ml of 0.1 N acid will be produced in tubes from which one of the growth factors has been omitted and at least 17 ml of 0.1 N acid will be produced after 66 hours' incubation at 37° C. in the presence of 2.0 micrograms of calcium pantothenate, 2.0 micrograms of nicotinic acid and 0.01 micrograms of biotin.

The blanks and maximal acid production obtained with a typical lot of peptone when substituted for casein are compared with the blanks and maximal acid production obtained with casein hydrolysate in Table 1.

TABLE 1

BLANKS AND MAXIMAL ACID PRODUCTION OBTAINED WITH CHARCOAL TREATED PEPTONE AND CASEIN HYDROLYSATE

Vitamin	Peptone		Casein	
	Blank	Maximum	Blank	Maximum
Biotin	1.5	18.1	1.74	17.55
Pantothenic acid	1.9	18.6	1.2	18.7
Nicotinic acid	0.84	19.0	0.6	18.3

All results are expressed as cc of 0.1 N acid produced after 66 hours' incubation, and are the averages of duplicate determinations.

TABLE 2

COMPARATIVE ASSAY VALUES FOR RAT URINE FOUND IN PARALLEL RUNS USING PEPTONE MEDIA AND CASEIN HYDROLYSATE MEDIA

Test	Value with peptone medium*	Value with casein medium*
Nicotinic acid	1.6	1.65
Calcium pantothenate	6.2	6.3
Biotin	0.02	0.02

* Expressed in micrograms per ml.