CLIII. THE APPROXIMATE DETERMINATION OF SPERMINE IN SINGLE HUMAN ORGANS.

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IN the writer's published work [Harrison, 1931] on the distribution of spermine in human tissues it was necessary to pool the smaller organs from a number of subjects in order to make a single estimation by the steam-distillation method. As a development of the subsequent study of Barberio's picric acid test for semen [Harrison, 1932] the method for estimating spermine in organs has been modified, so that approximate determinations of the amount in a single prostate, or in a pair of testes or in a single sample of human semen can be undertaken. The investigation is an extension of the work previously published, in the hope of throwing some light on the significance of the relatively large quantities of spermine in the average human prostate.

The modified method.

In brief, spermine and other substances are extracted from the dried material by trichloroacetic acid. An aliquot part of the cleared extract is precipitated with picric acid. The crude spermine picrate is converted into the hydrochloride, and then into the characteristic phosphate which is insoluble in 25 % alcohol. The spermine phosphate is separated, washed, dried and weighed.

The details are as follows. After mincing and weighing, the organ is heated on the boiling water-bath for several hours, absolute alcohol being added at intervals to hasten the drying process. It is then stored in a vacuum-desiccator as long as is convenient. After weighing, the dried organ is extracted with ether for 2 to 6 hours in a Soxhlet apparatus (when very fatty it is more satisfactory to weigh the organ after ether extraction). The residue is transferred quantitatively from the Soxhlet thimble into a glass-stoppered measuring cylinder of suitable capacity. 2.5 % trichloroacetic acid is added to a total of 10 volumes; *i.e.* each g. of dried (or of dried and ether-extracted) material is made up to a total of 10.0 cc. by addition of the acid. The mixture is thoroughly shaken at intervals, extraction being allowed to continue for 2 hours. Any contraction in volume is made good by addition of more acid, and, after mixing, the extract is decanted and cleared as much as possible by centrifuging or filtering or both. As a rule the final fluid is clear or only slightly opalescent. Its volume is measured, and an equal volume of saturated aqueous picric acid is added. The mixture is heated on the boiling water-bath till it is clear or only slightly opalescent. The flame is turned out and cooling allowed to proceed slowly to room temperature. (This makes the subsequent separation of the picrate less difficult.) After standing for a few hours (conveniently overnight), the picrate precipitate is separated by decanting and centrifuging (filtration is generally less satisfactory), and is roughly dried in air (*e.g.* in the incubator at 37° overnight). It is then dissolved in the minimum of boiling acid alcohol (concentrated HCl 7 cc., water 43 cc., alcohol to 200 cc.), and thrown into at least 10 volumes of acetone. The precipitate of crude spermine hydrochloride is filtered off, most conveniently through a Soxhlet thimble placed in a conical funnel which is mounted in a filter-flask, so that slight suction can be applied at the end of the process. After washing with acetone until free from picric acid, the funnel is transferred without delay to a suitable boiling-tube with side-arm. The precipitate

is dissolved in the minimum of hot water, a few cc. being added at a time followed by gentle suction. Phenol red is added as indicator, and then drop by drop a concentrated aqueous solution of diammonium hydrogen phosphate until a p_H of 7.0 to 7.2 is attained. After adding 1/3 vol. of absolute alcohol, the mixture is allowed to stand overnight at room temperature for the crystals of spermine phosphate to separate. The crystals are finally filtered off, washed with 25 % and then with absolute alcohol, dried and weighed. The following is an example of the calculation:

Fresh weight of minced prostate, 22.85 g.

Dry weight of same, 4.52 g.

Made up to total volume of 45.2 cc. with 2.5 % trichloroacetic acid.

Volume of filtrate, 30.5 cc., which corresponds to 3.05 g. of dried prostate.

Weight of dry spermine phosphate, 9.5 mg.

Therefore yield of spermine (as phosphate) = $\frac{9.5 \times 100}{3.05} = 311$ mg. per 100 g. dry prostate,

or $\frac{9.5 \times 100}{3.05} \times \frac{4.52}{22.85} = 61.5$ mg. per 100 g. fresh prostate.

Method for human semen.

A measured volume of semen is thrown into at least 5 volumes of absolute alcohol, mixed well and allowed to stand several hours (*e.g.* overnight). The precipitate of protein and spermine phosphate and other substances is filtered off with suction, washed with absolute alcohol and dried in air. The precipitate from each cc. of semen is made up to 5 cc. with 2.5 % trichloroacetic acid, and treated subsequently exactly as described above.

Notes on method.

The organ must be dried and (in most cases at any rate) extracted with ether. If the fresh organ is extracted directly with trichloroacetic acid, the extract contains fat and other substances in such a fine state of division that it cannot be cleared satisfactorily by centrifuging or filtering.

The crude spermine picrate generally comes out so finely divided that it is difficult or impossible to separate it by filtration. By redissolving with heat and slow cooling, followed by centrifuging, this difficulty is largely eliminated.

By the method above described the weighing of the picrate [see Harrison, 1931] can be avoided—an important point when dealing with such small quantities.

If the yield of spermine phosphate is relatively large, or if it is grossly pigmented, it is better to complete the precipitation by adding an equal volume of alcohol, then to redissolve the precipitate in boiling water, decolorise with charcoal and filter hot; phenol red is added to the filtrate, the reaction is adjusted if necessary, and 1/3 vol. of absolute alcohol is added as previously described [Harrison, 1931].

Several experiments were made on the time required for extraction by trichloroacetic acid. There was no significant difference in the yields when half the gland was extracted for 2, and the other half for 24 hours.

For the reason given below, the present method is not so accurate as the steam-distillation method. The latter is therefore to be preferred when working with large organs or with large numbers of pooled small organs. The present method is useful for single small organs, or as a preliminary test to discover roughly how much spermine an organ or other material contains.

DISCUSSION OF RESULTS.

The organs were all obtained *post mortem*; the cause of death is indicated in the Tables. To outward appearance none of the organs analysed was diseased; those cases in which abnormalities of the particular organs would be expected from the nature of the cause of death were excluded. The results have been arranged according to the subjects' ages. Some of the pancreases obtained for analysis were incomplete, portions being required for other examinations. When

examined microscopically the crude spermine phosphate finally obtained was seen, in the case of the prostates and human semen, to consist almost entirely of the typical lenticular crystals. The final material from testes contained a little amorphous matter. The crude phosphate from pancreas contained quite an appreciable amount of amorphous matter; the yields reported for spermine

Table I. *Prostates.*

Patient	Age years	Spermine (as phosphate) mg. per 100 g.		Weight of prostate, g.		Cause of death
		Dry gland	Fresh gland	Dried	Fresh	
W. S.	8	None detected		0.19	0.96	Cerebrospinal fever
L. W.	15	47	—	1.03	—	Malignant endocarditis
C. M. T.	19	188	—	1.34	—	Septic endocarditis
G. T.	31	427	—	1.47	—	Lymphadenoma
G. G.	31	346	66	3.93	20.7	Subphrenic abscess: septic meningitis
W. M.	35	326	—	2.20	—	Exophthalmic goitre
J. C.	38	625	117	2.49	13.3	Tuberculous bronchopneumonia
H. P.	39	1219	266	5.24	24.1	Pontine haemorrhage
F. B.	39	227	45	4.34	21.9	Addison's disease
R. T.	41	628	125	2.25	11.4	Carcinoma of pancreas
J. S.	42	86	15.6	2.46	13.5	Hodgkin's disease
E. R.	47	311	62	4.52	22.9	New growth and abscess lung
F. B.	49	1410	—	2.80	—	Gastric ulcer: broncho-pneumonia
R. T.	50	158	26	3.05	18.8	Familial jaundice: peritonitis
W. E.	55	663	—	3.00	—	Gastric ulcer
W. W.	57	1293	—	2.32	—	Gastrectomy: peritonitis
W. Wd.	59	506	98	6.07	31.3	Fractured skull
R. M.	60	965	—	3.04	—	Contracted kidney: uraemia
W. C.	60	989	182	4.06	22.1	Emphysema; cardiac failure
J. B.	61	1358	255	2.10	11.2	Rheumatic carditis
G. P.	65	557	145	13.72	52.7	Contracted kidney: uraemia
W. B.	66	695	131	3.26	17.4	Perforated gastric ulcer
W. N.	66	22	5.6	3.09	12.0	Renal tuberculosis and perinephric abscess
P. S.	66	930	173	4.69	25.2	Abdominal neoplasm
J. M.	67	387	82	2.16	10.2	Myocarditis
J. G.	72	302	61	2.75	13.6	Duodenal ulcer: arteriosclerosis
Average*:		582.6	109.1			

* Excluding W. S., age 8.

Table II. *Testes.*

Patient	Age years	Spermine (as phosphate) mg. per 100 g.		Weight of pairs of testes, g.		Cause of death
		Dry gland	Fresh gland	Dried	Fresh	
C. C.	32	102	16.0	6.24	39.9	Arthritis of hip: septicaemia
G. McC.	34	108	14.5	4.91	36.7	Chronic rheumatic carditis
C. S. C.	37	47	7.1	4.54	30.4	Chronic nephritis
C. H.	38	92	12.2	3.36	25.2	Carcinoma in lung
W. B.	42	23	3.5	4.20	27.3	Coronary thrombosis
H. B.	45	12	1.7	2.05	14.1	Phthisis
A. B.	46	58	9.8	4.11	24.4	Contracted kidney: uraemia
C. B.	50	71	12.0	5.36	31.7	Carcinoma of bladder
C. H.	57	70	11.6	6.39	38.3	Carcinoma of pancreas
T. B.	61	71	11.0	3.15	20.4	Peritonitis
H. S.	61	81	12.0	4.54	30.5	Pelvic neoplasm
R. C.	62	52	9.3	5.62	31.3	HCl poisoning
C. A.	65	65	10.0	3.83	24.7	Carcinoma of liver
W. B.	66	29	4.4	2.64	17.7	Perforated gastric ulcer
Average:		63	9.7			

Table III. *Pancreases.*

Patient	Age years	Spermine (as phosphate) mg. per 100 g.		Weight of pancreas, g.		Cause of death
		Dry gland	Fresh gland	Dried	Fresh	
T. E.	16/365	Present		0.31	1.09	Umbilical sepsis: bronchopneumonia
F. G.	4	303	61	2.24	11.1	Diarrhoea and vomiting
W. S.	8	253	54.5	6.14	28.5	Cerebrospinal fever
W. McS.	19	201	43	16.81	78.9	Acute rheumatic carditis
E. R.	26	123	27	6.78	30.9	Mediastinal lymphosarcoma
H. B.	37	151	50	12.55	38.1	Peritonitis
J. C.	38	123	45	22.69	61.6	Tuberculous bronchopneumonia
J. S.	42	230	40	12.59	72.4	Hodgkin's disease
H. G.	50	254	46	8.36	45.9	Perforated gastric ulcer
F. S.	50	156	30	15.27	79.9	Lymphatic leucaemia
W. E.	55	210	—	14.65	—	Gastric ulcer
W. C.	60	159	16.3	3.89*	37.8	Emphysema; cardiac failure
J. B.	61	105	13.5	13.08*	102.0	Rheumatic carditis
G. P.	65	117	15.1	8.13*	62.9	Contracted kidney; uraemia
J. G.	72	239	50.8	14.03	66.0	Duodenal ulcer; arteriosclerosis
Average:		187.4	37.9			

* Weight of dried organ less ether-soluble matter.

Table IV. *Miscellaneous.*

Material	Age years	Spermine (as phosphate) mg. per 100 g.		Weight of material, g.		Cause of death	Amorphous impurity in spermine phosphate
		Dried	Fresh	Dried	Fresh		
Stomach	15	127	22	22.9*	129.9	Miliary tuberculosis	Little
	50	101	18	22.1	127.8	Suppurative cholangitis: carcinoma of common bile-duct	"
	63	79	14	24.0	138.9	Duodenal ulcer	"
Small intestine	15	167	20	43.8*	364.1	Miliary tuberculosis	Fair quantity
	47	141	19	58.0*	428.6	Gastric ulcer: peritonitis	Little
	48	129	17	19.2*	150.4	Pneumonia	"
Large intestine	15	34	4.6	33.2	247.0	Miliary tuberculosis	Fair quantity
	47	65	8.6	25.2*	191.0	Gastric ulcer: peritonitis	"
	48	182	24	57.6*	444.1	Pneumonia	Little
Spleen	40	76	16	57.0*	275.6	Cerebral abscess: empyema	"
	53	111	26	20.5*	88.0	—	"
	55	52	13	24.8*	100.5	—	"
	65	112	22	12.7*	65.3	Carcinoma of common bile-duct	"
Uterus	42	31	8	11.5	44.5	Carcinoma of colon: peritonitis	Fair quantity
	49	46	11	22.1	92.5	Mediastinal growth	"
	54	18	5	11.4	41.3	Lobar pneumonia	"
Pairs of ovaries	41	26	4	2.0	12.8	Suppurative perinephritis	Considerable
	54	11	2	1.1	4.4	Lobar pneumonia	"
Thyroid	49	20	5	2.0	7.8	Mediastinal growth	Fair quantity
	57	15	5	5.8	15.6	Duodenal ulcer	"
	65	7	2	3.5	13.8	Suppurative diverticulitis: gangrene of colon	"
Semen		Date (1932)	mg./100 cc.		cc.		
	38	26. ii	233	—	3.0	Same individual	
		4. iii	112	—	3.5		
		15. iii	268	—	3.3		
		29. iv	170	—	4.5		
		14. vi	160	—	3.2		
		21. vi	154	—	2.7		
	25	—	63	—	1.0	Healthy individual	

* Weight of dried organ less ether-soluble matter.

phosphate are therefore definitely too high, though probably not more than + 10 %. With some of the other organs reported in Table IV the proportion of amorphous matter was still greater (see last column of Table IV), but the figures at least indicate that in all these organs, spermine, though present, is not in excess of the amounts found generally elsewhere; they extend the contrast between the yields from prostates and other organs respectively, because several of the organs in Table IV had not been examined in the previous investigation.

The amorphous matter in the crude spermine phosphate finally separated was mainly protein or cleavage products of protein.

The average yield from prostates (Table I) in this series (109.1 mg. per 100 g. of 17 fresh prostates) is of the same order as the previous yield [Harrison, 1931] of 130 mg. per 100 g. from 1594 g. of pooled organs collected under alcohol. But the individual results vary enormously (5.6 to 266 mg.), and no correlation is apparent between yield and either the patient's age, or the size of the gland or the type of disease causing death. The range is so great as to make it unlikely that similar estimations in diseases of the prostate would be of any value. It would seem that the quantity of spermine in the prostate is fortuitous. Possibly any excess in the body is excreted by this gland. Certainly the results do not suggest that spermine has any particular functional significance in connection with the prostate or with reproduction.

It has been possible to make only a few determinations on normal human semen (Table IV). The results vary considerably; this is strikingly shown in the series of observations on one individual over a period of 4 months. Since the spermine of semen is probably mainly if not entirely derived from the prostate [Harrison, 1931] these findings are to be expected.

The spermine contents of 14 individual pairs of testes (1.7 to 16 mg., Table II) vary much less than those of prostates. They are of the same order as previously reported for pooled testes and are not above the amounts found generally in the organs of the body.

The results for 13 individual pancreases (Table III) vary from 13.5 to 61 mg. per 100 g. fresh gland, with a mean of 37.9 mg., as against the value of 16.1 mg. previously reported for 3361 g. of pooled pancreases. As explained above the present figures are a little too high in view of impurities.

SUMMARY.

1. A method is described for the approximate determination of spermine; this is useful in examining single small organs or a single sample of semen, and as a preliminary test to discover whether material contains significant amounts of spermine.

2. The spermine contents of individual normal prostates obtained *post mortem* vary enormously. Though the average yield is high, confirming previous work, the conclusion is drawn that the amount is fortuitous, possibly representing an excretion of excess from the body. The results throw no light on the possible functional significance of spermine.

3. The approximate spermine contents of a few more human organs are reported; they are of the same order as found in all organs other than the prostate.

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REFERENCES.

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