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STUDIES WITH A RADIOACTIVE SPINAL ANAESTHETIC

BY

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The need for information about the fate of a spinal anaesthetic after its introduction into the spinal theca was early recognized (Miller, 1901), but many years elapsed before the problem was seriously studied by a number of workers, including Stout (1929), Küstner and Eissner (1930), Koster *et al.* (1936, 1938, 1939), Bullock and Macdonald (1938), and Shields (1942). In the main these workers agreed that after the intrathecal injection of procaine there was a rapid initial drop in local concentration with a subsequent period of gradual decrement.

Evidently an anaesthetic departs from the subarachnoid space, but what is its route of departure? There are various theoretical possibilities:

(1) Cephalic transit in the spinal cerebrospinal fluid to the cranial subarachnoid space with possible absorption by the cranial arachnoid villi.

(2) Absorption by spinal cord and/or nerve roots with or without a period of "fixation" in these tissues.

(3) Transport *via* the lymphatic drainage of the spinal theca.

(4) Absorption into the venous drainage of the spinal theca either directly or *via* spinal arachnoid villi (Elman, 1923).

(5) A combination of any of the foregoing.

It is proposed to describe a study of the fate of a spinal anaesthetic with special reference to its ultimate distribution among the tissues of the body and the routes by which it leaves the spinal theca.

Method of assay and anaesthetic employed

The method of assay had to satisfy two main criteria: (1) indifference to the nature and amount of the diluting media; (2) a high degree of sensitivity; the former since it was proposed to undertake analyses both in body fluids and various tissues, and the latter since it was not intended to employ disproportionate doses of the anaesthetic. Of chemical methods the diazo-reaction is probably one of the most sensitive for procaine assay: Küstner and Eissner (1930) and Willstaedt (1934) claimed sensitivities to 0.01 and 0.005 mg. respectively for their techniques, while Bullock and Macdonald (1938) asserted that concentrations below 0.02 per cent were beyond their powers of estimation. It was anticipated that the tissue concentration after a normal spinal anaesthetic in the cat would usually be below any of the limits given above, while the gross excess of tissue bespoke laborious separation techniques in which the accumulated error promised to be large even if enough drug were present to render assay possible.

A tracer technique seemed, therefore, to be indicated. Although theoretically any of the atoms of the procaine molecule is open to substitution by its radioactive counterpart, such a procedure was found impracticable when this research commenced because of limited half life, uncertainty of supply, difficulties of assay, or synthesis with the elements normally present. It was therefore decided to introduce some other element into the benzene nucleus without radically changing the pharmacology of the anaesthetic. The halogens were the obvious choice, and preliminary experiments showed that of these Br^{82} was the most satisfactory. Procaine was accordingly brominated by the method of Morel, Leulier, and Denoyel (1929), the necessary hydrobromic acid being prepared as described by Howarth (1948). In this description the author made use of calculated quantities of procaine in the bromination stage, but subsequent experience has shown the reaction to be sufficiently easily controlled to render this unnecessary.

The method was as follows: the concentrated hydrobromic acid having been obtained, 3 or 4 c.c. of 100 vols. H_2O_2 were added to it followed by a knife

point of procaine. The solution in the tube promptly developed a yellow tinge. Further procaine was then added and the coloration disappeared, to return again after a minute or two when more procaine was added. This procedure was repeated until the reaction was completed. In this way the speed of bromination was increased and the necessity for time-consuming estimations eliminated.

The bromine for these experiments was obtained either as KBr from the cyclotrons of Liverpool or Cambridge or as ethylene dibromide from the small or large pile at Harwell. When the small pile was employed 3–6 ampoules each containing 10 c.c. of ethylene dibromide and 5 per cent aniline (Le Roux *et al.*, 1939; Lu and Sugden, 1939) were irradiated, the total activity per ampoule being of the order of 200 millicuries. With the large pile (factor of 10) 1–2 c.c. were irradiated. After this the bromine was extracted as KBr by the Szilard-Chalmers technique (1934).

The dibromoprocaine hydrobromide $C_{13}H_{18}Br_2N_2O_2$, HBr (DBP hydrobromide) thus obtained was converted to DBP hydrochloride either by the method of Morel *et al.* (1929) or latterly by precipitating the base with N/10 NaOH, dissolving the latter in ether, separating and precipitating the hydrochloride with a stream of HCl gas. The entire operation from KBr to DBP hydrochloride occupied about three hours.

Assay was carried out by means of a standard Dynatron X 200 scaling unit and a counter designed to take 10 c.c. of liquid. Body fluids were assayed directly; tissues were broken down in alcoholic lithium hydroxide (Howarth, 1949a) and suitable dilutions of the suspension employed.

Preliminary studies

Three preliminary observations were needed:

(1) Investigation of the spinal anaesthetic properties of DBP.

(2) An assessment of the accuracy of the author's method of radioassay.

(3) Study of the fate of brominated benzene rings in the body.

(1) *DBP as a spinal anaesthetic*

Morel *et al.* (1929) in their description of DBP did not include studies of its applications in spinal anaesthesia.

The anaesthetic properties of DBP were examined as follows: six cats were premedicated with atropine and lightly anaesthetized with ether; each received 10 mg. DBP, HCl in 1 c.c. H_2O at 40° C. by lumbar puncture and the ether administration was then discontinued.

All showed relaxation of the anal sphincter (lower sacral block), loss of knee jerks (lumbar block), but no obliteration of the elbow jerk (lower cervical roots). The hindlimbs were atonic and could be placed in exaggerated postures. On recovering consciousness all the animals appeared paralysed from the lower costal margins downwards; they dragged their hindlimbs. They displayed no interest in violent stimuli administered to their hindlimbs, contrary to the effects of similar stimuli to their forelimbs. In one animal there was evidence of some degree of intercostal paralysis as shown by reduced thoracic and increased diaphragmatic excursions. All the animals recovered completely and walked perfectly within 24 hours. None showed tail droop after this period. It was concluded that DBP was a powerful spinal anaesthetic but with limited usefulness owing to its low solubility. Although only six cats were examined under these controlled experimental conditions, the author has administered DBP to some 200 cats and six monkeys without producing any persistent ill effects except in four cases, and in at least two of these a persistent paraplegia was due to haematomyelia after lumbar puncture.

(2) *Accuracy of radioassay*

In order to investigate this an anaesthetized cat received 0.5 c.c. of a DBP, HCl solution by lumbar puncture. Six samples of cerebrospinal fluid were collected and each assayed by the author and by Dr. K. Bullock, who kindly undertook the chemical estimations. The basis of the latter was diazotization and coupling followed by photocolorimetry. Standards prepared gravimetrically were also separately assayed. The results showed a satisfactory agreement (Table I).

TABLE I

CONCENTRATION OF DIBROMOPROCAINE HCL IN SUCCESSIVE SAMPLES OF CEREBROSPINAL FLUID (G./100 C.C.)

Sample Number	Estimation Diazo Dr. Bullock	Estimation Radioassay Author
1	0.043	0.050
2	0.037	0.041
3	0.022	0.024
4	0.011	0.012
5	0.005	0.0041
Vol. of C.S.F. assayed ...	0.1 c.c.	0.02 c.c.

Estimation of a standard solution (g./100 c.c.)

Gravimetric	0.015
Diazo assay	0.018
Radioassay	0.016

In later experiments closer agreement has been reached.

(3) *Fate of DBP*

On account of its great stability it is unlikely that DBP suffers removal of its nuclear halogen atoms during its sojourn in the body (Fromherz, 1928; Williams, 1947). Nevertheless, from the point of view of laboratory manipulation, stability of a halogen attached to a benzene ring within an organic compound is influenced by the arrangement of the various groupings about such a ring. Thus it was expedient to investigate the drug from this point of view, since the removal of a large proportion of tagged atoms would severely reduce their value as a tracer for this molecule.

The method was as follows:

An anaesthetized cat received by the preaxial vein of the forelimb 0.1 g. DBP in 20 c.c. water, injection being made slowly so as to avoid cardiac or respiratory depression. After two hours the animal was sacrificed, the abdomen opened, and the urine aspirated from the bladder; 1 c.c. of the urine was placed in each of five small tubes. To each of these were added 2 c.c. of a NaBr solution to serve as a carrier, and to four of them 0.5 c.c. of a saturated solution of $AgNO_3$. The precipitate of AgBr was filtered off and washed thoroughly, the filtrate being made up to 10 c.c. and subjected to radioassay. The figures obtained were compared with those derived from the tube containing no $AgNO_3$. The results (Table II) showed that most of the bromine atoms in the urine did not exist as ionizable bromine, but as bromine in organic combination. The last two lines of the Table indicate that the anaesthetic itself, prepared by the methods described, did not contain ionizable bromine.

It has been shown recently that even after five days the urinary bromine exists chiefly as bromine in organic form.

TABLE II

This table shows that the radioactivity of urine after intravenous dibromoprocaine hydrochloride was not reduced by treatment with sodium bromide (carrier) and silver nitrate followed by filtration. The radioactive bromine atoms have not been precipitated by the silver nitrate; therefore the bromine is organically bound. The last two lines show that there is no ionizable bromine in the anaesthetic itself.

Exp. No.	Contents of tube (c.c.)				Standardized counts per min.
	Urine	Silver nitrate	Sodium bromide	DBP HCl soln.	
1	1	0	2	0	684
2	1	0.5	2	0	642
3	1	0.5	2	0	651
4	1	0.5	2	0	656
5	1	0.5	2	0	684
6	0	0.5	2	1	160
7	0	0	2	1	165
	Statistical error ± 4% S.D.				

Note on interpretation of results

The concentration of DBP hydrochloride in an aqueous solution was estimated by comparing its counting rate with that of a standard of known concentration. In the tissues, however, procaine and probably DBP is broken down into a number of products (Burgen *et al.*, 1948; Goldberg *et al.*, 1943). In the present work the results of comparison with standard solutions of DBP are expressed as μg. of the latter per g. (or c.c.) of tissue. This should be understood as that amount of DBP, HCl which would be present if all the benzene nuclei were incorporated in molecules of DBP.

Distribution in Body Fluids

In this series of experiments it was decided to investigate the levels of DBP obtaining in the C.S.F., blood, and urine after intrathecal injection.

Method

Cats anaesthetized with pentobarbitone received various doses of DBP, HCl by lumbar puncture. The needle was left *in situ* with stilette in position, but at fixed time intervals the stilette was withdrawn, and, after permitting a few drops of C.S.F. to escape, the inside of the butt of the needle was cleaned with a spill of filter-paper and 0.02 c.c. of C.S.F. was collected in a blood pipette. Blood was taken at similar time intervals from a cannula inserted into the femoral or carotid artery. Urine was obtained by suprapubic cannulation of the urethra, the cannula being tied in position and led by way of a narrow rubber tube into a small collecting phial. The phial was changed at 15-min. intervals, the last of the urine being expressed by gentle pressure over the bladder just before the change of receiver. Further specimens of C.S.F. were obtained from the cisterna magna by means of a hypodermic needle passed through the atlanto-occipital membrane, the volume removed at each sampling being 0.1 c.c. The various specimens obtained were subjected to radioassay.

Results

The graph (Fig. 1) shows the results obtained from one experiment. The main features are the rapid initial decline of the drug concentration in the C.S.F., followed by a less steep phase, the rapid rise of urine concentration followed by a slower fall, and the low steady blood level of drug. The concentration within the cisterna was usually so low as to render accurate assay valueless.

Five animals were studied in detail and the curves for urine, C.S.F., and blood found to be similar in form for each animal though there was some individual variation; thus though the decline of the concentration of C.S.F. was always rapid, the actual rate of fall varied. There appeared to be

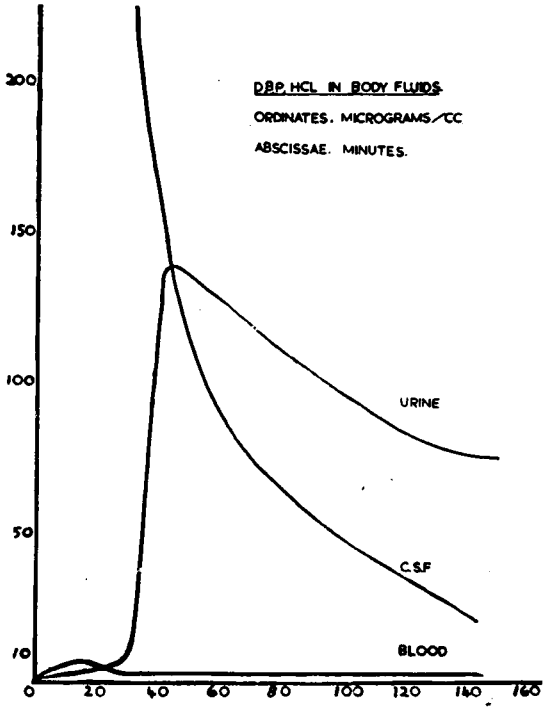

FIG. 1.—Dibromoprocaine levels in body fluids after spinal subarachnoid injection.

no simple relationship between rate of decrement in concentration, dose of drug, and weight of animal. The " bump " on the blood level seemed to correspond to the period of maximum decline of the C.S.F. concentration. The form of the urine curves presented no striking differences except that the peak level varied both as regards time and maximum height.

TISSUE DISTRIBUTION

The observation that DBP rapidly leaves the theca, appears in the urine in high concentration, and maintains a persistently low blood level made it of interest to study the distribution of the drug among the body tissues. A preliminary note of this study has been published (Howarth, 1949b).

Methods

After subarachnoid injection

The cats were anaesthetized with pentobarbitone, the carotid artery cannulated, and a spring clamp placed upon the vessel proximally. The bladder was catheterized and a dose of DBP administered by lumbar puncture, the needle and stilette being left *in situ*. The presence of spinal anaesthesia was confirmed and the animals left for a specific period. Fifteen minutes before autopsy the bladder was emptied and the catheter clipped so as to collect within the bladder a 15-minute specimen of urine. Preparation was now made for autopsy, and a sample of blood (1–2 c.c.) was taken in a small tube and shaken with a few crystals of sodium citrate in order to prevent clotting. The clamp was removed from the catheter and the urine collected. The stilette was withdrawn from the lumbar puncture needle and, after allowing two or three drops of C.S.F. to escape, 0.02 c.c. of C.S.F. was collected in a blood pipette and discharged with washing into about 4 c.c. of water.

Autopsy

Autopsy was performed in the usual manner, special efforts being made to prevent loss of blood.

The spinal cord was removed complete with membranes whilst the animal was still alive, so as to reduce post-mortem absorption of DBP remaining in the cerebospinal fluid. The dura mater was stripped from the spinal cord and the roots of the cauda equina divided close to the cord and retained for assay. One centimetre of cord was removed from the lumbar region at the site of introduction of the DBP. Neither roots nor cord were cleaned, and consequently some of the administered anaesthetic adhered to their surfaces.

Removal of samples

In removal of specimens from an organ adherent blood was removed from its surface and a small section excised, free blood being gently swabbed from the cut surface. An endeavour was made to remove the same portion of each organ in each experiment. A list of samples is appended with relevant notes.

Cord and roots (see above).

Sciatic nerve in its entirety.

Medulla oblongata from the level of the atlas to the lower border of the pons.

Spleen about half an inch from its left-hand extremity.

Liver. A portion from the middle of the anterior surface of the right lobe.

Bile aspirated from gall-bladder after liver specimen had been removed.

Kidney. This was incised along the convexity and the organ decapsulated by pressing it through the incision. The kidney was divested of cortex with a razor blade starting at the attachment of the ureteric pelvis which marks the cortico-medullary junction. Cortex and medulla were assayed separately.

Muscle. The bellies of the left hamstrings.

Lung. Lower quarter of the right lung.

Gut contents. These consisted of the contents of the third part of the duodenum and were extracted by massage of the isolated length of gut held over a watch glass.

Gut. A portion of the second part of the duodenum.

Thyroid and testis (or ovary) in their entirety.

Skin. A portion from the scalp shaved prior to autopsy.

TABLE III

TISSUE/BLOOD RATIOS (*Ct/Ca*) AFTER INTRATHECAL INJECTION OF DIBROMOPROCAINE HYDROCHLORIDE

Weight of cat (kg.)	3.6	2	3.5	2.1	3	2.5	2.25
Dose (g.)	0.007	0.007	0.025	0.007	0.008	0.03	0.005
Time after injection (hr.)	1	1.23	2.5	3	21	39	20
Blood*	3.36	5.41	3.34	2.73	1.31	2.86	0.78
Spleen	0.26	0.25	0.24	0.30	0.50	0.22	2.51
Medulla	0.091	0.11	0.074	0.13	0.30	0.12	0.25
Sciatic nerve	0.27	0.37	0.2	0.48	0.84	0.31	0.63
Muscle	0.11	0.1	0.049	0.067	0.13	0.074	0.19
Lung	0.73	0.52	0.47	0.67	0.80	0.44	0.50
Skin	0.5	0.32	0.31	0.63	0.93	0.58	1.29
Thyroid	0.49	0.27	0.19	0.4	0.80	—	—
Testis	0.53	1.5	0.28	0.54	0.6	0.30	—
Gut	0.34	3.1	0.48	0.56	0.6	0.29	0.59
Kidney cortex	8.43	9.3	6.2	7.2	1.3	0.36	3.40
Kidney medulla	6.4	4.3	2.6	2.7	1.5	0.88	1.80
Liver	2.3	2.8	1.7	1.4	1.1	0.34	1.35
Bile	6.73	26.6	7.2	8.6	9.8	0.67	5.7
Urine	83	27	24	133	4	0.8	15.7
Cord*	38.3	26	26	8.4	0.64	0.6	0.55
Roots*	163	94	76	76	1.6	0.8	0.87
C.S.F.*	103	37	19.7	22	1.3	1.7	—
Duodenal contents*	6.76	92	7.2	18.3	None	3.6	—
Error	3–4%						

* Expressed as μg./g.

Estimation

Specimens were placed on watch glasses on a tray over which a wet cloth was suspended in order to reduce evaporation. The specimens were weighed within a few minutes of removal from the body, the smaller organs—i.e., thyroid, nerve roots, and ovary—being weighed immediately. Assay was undertaken as described above.

Results

Table III shows the results obtained from seven animals. The drug concentrations found in C.S.F. cord, roots, and duodenal contents were not directly dependent upon blood concentration, and these were expressed as μg./c.c. or μg./g. of tissue. The rest of the tissue concentrations were expressed as the ratio between mass of drug per gramme of tissue and mass of drug per c.c. of blood (*Ct/Ca* ratio).

The blood contained in the vascular bed of the organ under consideration could not be satisfactorily removed. Thus each assay included an unknown modicum of drug in the blood of its vascular bed. This capillary volume could not be expected to remain constant in different animals. Furthermore, the doses of drug and the time intervals before autopsy were also varied, and hence consistent results were not anticipated. This difficulty has been mentioned by Wallace and Brodie (1937) in their estimations of tissue iodides and thiocyanates.

Of all the tissues examined, apart from those in direct contact with the drug in the C.S.F., only liver and kidney showed a tissue concentration above that obtaining in the blood (tissue/blood ratio). Of these the kidney values were much the higher, and in the majority of the assays the cortex showed a higher concentration than the medulla. The concentration in the duodenal wall appeared puzzling and erratic until it was found that the bile contained an appreciable amount of DBP. It is interesting to note that both blood and C.S.F. contained detectable amounts of DBP 39 hours after its injection.

The concentrations found in the cord and roots show special features. In all but one experiment, the drug concentration was lower in the cord than in the C.S.F., but the roots always contained a higher concentration of drug than the cord. It appeared that the roots and not the cord were capable of concentrating the drug from the C.S.F. in which they were bathed.

The medulla oblongata showed a very constant tissue/blood ratio, and such constancy would not be expected had the drug ascended within the spinal subarachnoid space to any significant extent. The tissue distribution after intravenous

TABLE IV

TISSUE/BLOOD RATIOS (*Ct/Ca*) AFTER INTRAVENOUS ADMINISTRATION OF DIBROMOPROCAINE HYDROCHLORIDE

	Values for *Ct/Ca* after intravenous injection					
Weight of cat (kg.)	2.5	3.75	2.5	2.6	2.5	3
Dose (g.)	0.036	0.17	0.092	0.05	0.078	0.12
Time after injection (hr.)	1.20	70	4	2	4	2
Spleen (0.25)	0.27	0.49	0.28	0.2		
Medulla (0.11)	0.14	0.19	0.14	0.12	0.18	0.1
Sciatic nerve (0.33)	0.4	0.5	0.33	0.38		
Muscle (0.08)	0.14	0.11	0.11	0.09		
Lung (0.57)	0.81	0.75	0.71	0.4	0.6	0.55
Skin (0.47)	0.53	0.87	0.30		0.57	
Thyroid (0.34)	0.34		0.53	0.28	0.5	
Testis (0.41)	0.78	0.65	0.71	0.46		
Gut	0.93	0.31	0.08	0.55		
Kidney	5.75	1.23	1.71	2.4		
Liver	2.6	0.38	0.32	1.23	1.06	1.46
Bile	None	0.66	25.6	6.02		
Urine	39	0.27	30.2	30.6		
Cord	0.14	0.17	0.05	0.045	0.12	0.06
Roots	0.27	0.44	0.24	0.22	0.41	0.26
Blood (mean level 3.54 μg./g.) ...	22.1	4.52	33	43.9	11.4	38.8

Figures in parentheses are mean *Ct/Ca* ratios after intrathecal injection.

injection was also studied in order to ascertain whether any difference existed between root and cord concentrations, and to study the effects on tissue/blood ratios of higher blood levels.

The drug was injected into the preaxial vein of a forelimb and assays of organs made at various intervals. The results are tabulated (Table IV). It will be noted that four animals were studied in detail, but that estimations were made of tissue/blood ratios for cord and roots in six animals. For convenience of reference the mean tissue/blood ratios for the intrathecal analyses are included in the left-hand column. In this series of means, the 21-hour-period results are not included; means have not been provided for gut, bile, liver, kidney, or urine, since these results were variable, depending largely upon the state of the animal and the time interval between administration and sampling. In a similar way no means are tabulated for cord and roots since these values are mainly dependent on the concentration of drug in the theca.

The results show that even after intravenous injection the roots reveal a higher concentration of drug than the cord. On the whole the tissue/blood ratios after intravenous injection show a slight general increase, but in view of the relatively enormous blood levels it is doubtful if this increase has any real significance.

The concentration of the drug in the contents of the descending colon was assayed and also the concentration obtaining in the bone marrow of the femur. The values were of the order 2 μg./g. and 0.23–0.34 (tissue/blood ratio) respectively.

Only two observations were made and no assays were undertaken of the entire mass of faeces; the results merely indicated that some of the drug was excreted *via* the gut.

The concentration of drug in the cisterna magna was usually insignificant except in one case where the concentration rose to 1.9 μg./c.c. after 1½ hours. It was of interest, since assays of the cervical cord were planned, to discover the origin of this drug in the cisterna; some had undoubtedly risen from the lumbar regions, but it was possible that some had been secreted by the choroid plexuses.

DBP and the blood/C.S.F. barrier

DBP was administered by the preaxial vein of the forelimb, and specimens of C.S.F. were taken at specific intervals from the cisterna magna. Blood samples were taken at similar periods from the right femoral vein. A graph of such an experiment (Fig. 2) shows that DBP circulating in the blood in sufficient concentration was able to pass the choroid plexus.

Since it had been ascertained that DBP passes rapidly into the blood stream and its distribution

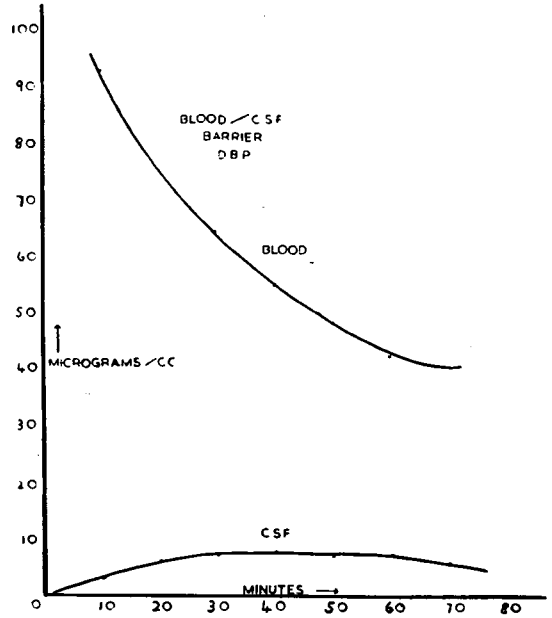

FIG. 2.—Dibromoprocaine appears in low concentration in the cerebrospinal fluid after intravenous injection of 132,400 μg. of DBP, HCl. (Cat 2.5 kg.)

among a number of organs had been studied, it became of interest to investigate the route by which it left the spinal theca.

The possibility of lymphatic drainage will first be considered.

Lymphatic drainage of the subarachnoid space

Since the lymphatics draining the region of the lumbar theca ultimately enter the thoracic duct, it was decided to cannulate this duct above the diaphragm. This was effected in the following manner: Under nembutal anaesthesia the lower part of the thoracic aorta was exposed by the appropriate rib resection, haemostasis being obtained by previous ligation of the ribs in front and behind the proposed resection, and afterwards by diathermy. When the thorax was opened the animal was maintained with artificial respiration, and the lungs were packed away from the aorta. The exposure was extended caudally into the costophrenic angle, and by depressing the cupola of the diaphragm a view of the aortic orifice was obtained. The parietal pleura was dissected away from the right side of the aorta until the thoracic duct appeared like a white thread on the pink aortic wall. The duct was ligated and operations discontinued for 30 minutes.

On recommencing the operation, the distended cisterna chyli could be seen at the aortic orifice. The pleura was removed from the cisterna chyli and a loose ligature cast around the upper part of the latter. A large-bore, curved, and blunted intramuscular needle was used as a cannula, and this was driven by steady pressure into the cephalic end of the cistern and tied in position. A male connector bearing a short length of cycle valve tubing was fixed into the cannula and the wound closed so as to leave an inch of tubing protruding through the chest wall. The chyle flowed freely at first, but the cannula frequently became obstructed. It was then necessary to empty the cistern by gentle aspiration with a syringe. Fig. 3 shows the exposure and cannulation of the thoracic duct.

Introduction of DBP

The muscles of the back are drained in part by the lymphatic channels which enter the thoracic duct (Gray, 1946), and introduction by lumbar puncture was thus unsuitable as a routine procedure since leakage into the muscles of the back was possible; in order to avoid this the theca was exposed where it passes over the sacral promontory and a narrow-bore cannula introduced into the subarachnoid space and tied in position.

Samples of chyle and C.S.F. were taken at frequent intervals after introduction of DBP, HCl. Since the maximal fall of C.S.F. concentration occurred over the first 30 minutes (Fig. 1) it was decided to limit the observations to this period.

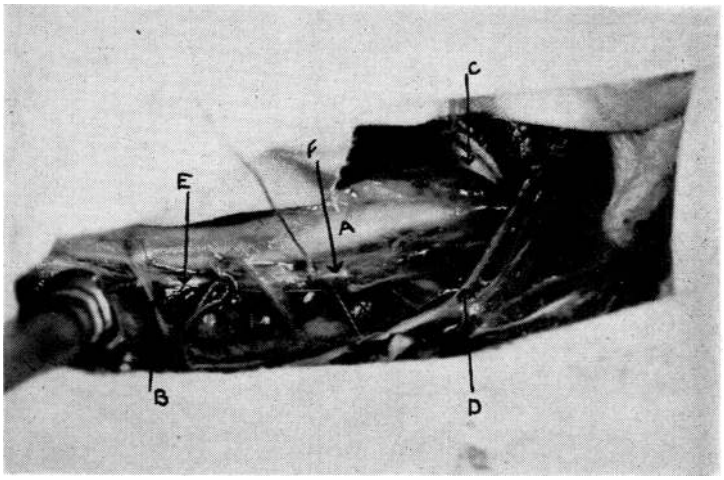

FIG. 3.—Supra-diaphragmatic cannulation of thoracic duct. The chest has been opened: A, aorta; B, intercostal artery; C, crus of diaphragm; D, splanchnic nerves; E, curved cannula and rubber connector; F, tip of cannula in thoracic duct.

Results

Fig. 4 shows that the C.S.F. concentration fell rapidly notwithstanding the new method of introduction and the extensive surgery. All six animals thus investigated showed this. The quantity of drug excreted by the thoracic duct was in all experiments too low to be represented on the graph. Table V gives the figures for the experiment relating to curve 2 on Fig. 4. It is evident that the total excretion by the thoracic duct is not a considerable factor in the reduction of drug concentration in C.S.F.

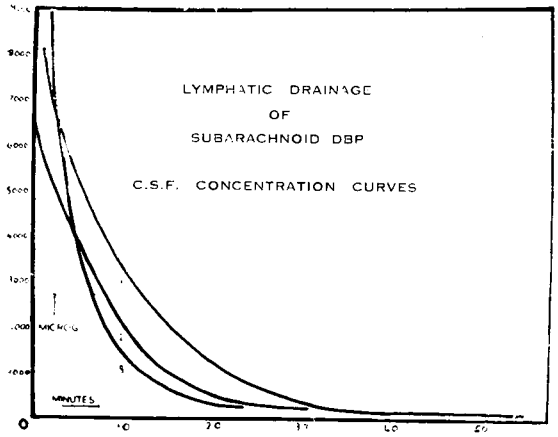

FIG. 4.—The rate of fall of the concentration of dibromoprocaine in the cerebrospinal fluid is rapid despite cannulation of the thoracic duct. The concentration of drug in the duct is too small to show on this scale.

In a comparable animal receiving the same dose but administered by lumbar puncture, the total drug excretion in the chyle attained 24.3 μg. in 27 minutes, during which time the C.S.F. concentration fell from 8,620 to 245 μg./c.c.

TABLE V

CAT ♂2.4 KG. DOSE BY SACRAL CANNULA: 12,000 MICROGRAMMES

Time after injection (min.)	DBP in C.S.F. (μg. per c.c.)	Chyle	
		Volume (c.c.)	DBP (μg.)
1	7,800	0.40	0.37
4	5,500	0.30	0.27
8	4,300	0.32	0.26
12	2,900	0.50	0.44
16	1,700	0.40	0.32
21	1,100	0.35	0.63
33	260	0.50	1.14
55	160	0.25	0.31

Total volume of chyle = 3.02 c.c.
Total excretion = 4.24 μg.
= 0.035% of total dose

Passage of DBP into the spinal cord

The possibility of absorption of drug by the intrathecal neural elements was next considered. Previously (Table III) it has been shown that the roots appear to concentrate the drug from the C.S.F. but that the cord does not. There is no information about the concentration within the cord substance itself, and the values obtained include an indeterminate quantity of anaesthetic clinging to the surface. The technique first employed was that of radio-autography.

Method

It is a *sine qua non* in radio-autography that the position of the active substance in the specimen must not change as a result of the application of histological reagents, but though the final form of DBP in the nervous tissues was unknown the difficulties inherent in the solubility of DBP, HCl itself were fully realized. A rapid fixation of the drug in the tissues was necessary to ensure that diffusion would not occur after death.

In an anaesthetized cat laminectomy was performed over the lumbar enlargement. The dura was held apart by small haemostats. The C.S.F. was allowed to escape and the knee jerks elicited. A pledget of wool soaked in 0.5 per cent (w/v) DBP, HCl in water or Dale's Ringer at body temperature was placed on the lumbar enlargement, absent knee jerks indicating the onset of anaesthesia. The pledget was replaced every five minutes until five changes had been made. Five minutes after the last application the spinal cord was clamped above and below the site of medication and the intervening length of cord with its attached roots removed *en masse* from the vertebral canal. The cord was severed just beyond the site previously occupied by the wool, and using the upper clamp as a handle the remainder of the cord was plunged forthwith into a Dewar flask containing liquid air. The cord was frozen solid and the anaesthetized portion isolated with an ampoule saw, placed upon the stage of a freezing microtome, and sections of 30 μ were cut. The sections were laid in the centre of small cells constructed upon coverslips and flattened with a spatula. The coverslips were placed immediately on a metal plate kept "cold" by liquid air. The coverslips were then transferred to small squares of Ilford no-screen *x*-ray film, resting upon small circles of thicker glass. Each cell was placed in a typewriter ribbon box and stored in the refrigerator for a week. A specimen was inspected from time to time to ascertain that it was still frozen, to develop a film, and to inspect the autograph produced at various time intervals. Subsequently all were developed and the sections returned to the refrigerator during the

development period when the coverslips bearing the sections were superimposed on the negative and the area of darkening in the latter ascribed to the relevant parts of the cord.

Figs. 5A, B, and C are autographs obtained by this technique. They exhibit a zone of radioactive anaesthetic surrounding and outlining the periphery of the cord. Within this zone are the nerve roots cut in transverse section, and to demonstrate

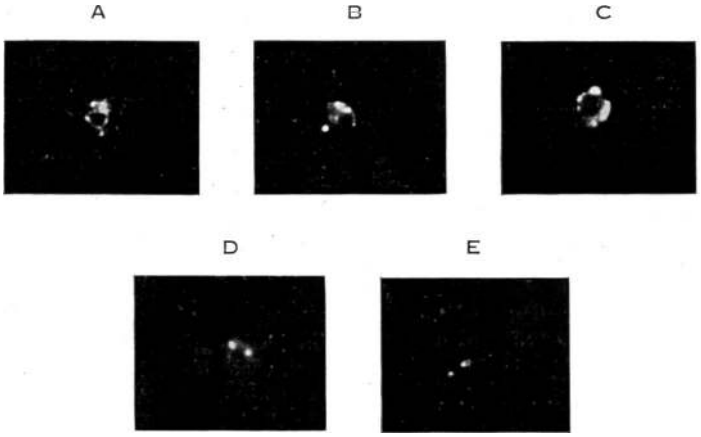

FIG. 5.—Radioactive anaesthetic around a number of cord sections. In D and E most of the anaesthetic has been washed away, but that in the roots remains. These autographs were made by a crude technique before the advances of Bélanger and Leblond (1946) and Pelc (1947). For details see text.

them a number of sections were rinsed in water before placing them on the coverslips. Figs. 5D and E show the results of this manœuvre, the roots containing a higher concentration than the cord and revealing themselves as intense white spots.

Results of these autographic studies should be interpreted with caution since the edge of the cord could not be related to the edge of the blackened area exactly, and the apparent absence of drug in the centre of the cord may simply indicate a concentration inadequate to affect the film (Hamilton, 1942).

Direct estimation of penetration

It was desirable to determine by some more sensitive method whether any drug had penetrated into the depths of the cord.

Method

In a number of anaesthetized cats, cannulae were inserted into the right common carotid artery and the sacral subarachnoid space. A dose of DBP was introduced by the sacral cannula. A hypodermic needle was passed into the cisterna magna. After a specific interval a specimen of blood was taken from the carotid artery and samples of C.S.F. from both the cisterna and the lumbar sac. After laminectomy the cord and membranes were removed from the upper cervical to the lower sacral region, bleeding being controlled by diathermy and haemostats. The cervical end of the cord was held in artery forceps and the entire cord lowered slowly into a Dewar flask containing liquid air. The frozen cervical and lumbar enlargements were isolated. Specimens were now removed from the centre of the lumbar enlargement. The cervical portion was returned to the liquid air to await attention. The lumbar enlargement, wrapped in gauze and lint, was permitted to thaw until it reached a consistency suitable for cutting. The wrapped piece of cord was held in the hand and the upper half-inch of dura was incised longitudinally on the ventral and dorsal aspects, the two flaps so formed being reflected over the rest of the cord by retraction with toothed forceps. The underlying pia was heavily contaminated with radioactive DBP, and it was essential to avoid contact between this and the tissue.

The pia and periphery of the cord were removed by making thin longitudinal tangential slices all round in such a way that the ends of the slices overlapped. The knife was washed and dried before each cut and the cord returned to the liquid air from time to time in order to maintain its frozen state. In this way a central core of relatively uncontaminated cord was obtained which was severed near the projecting uncut cord.

A chrome steel cylinder having at one end a razor-sharp cutting edge had been prepared and was placed with its cutting edge against the uncontaminated cord. By gentle screwing movements the cylinder was driven through. When a suitable distance had been traversed the cord was incised around the metal cylinder just proximal to its cutting edge. The rest of the cord was gently pulled away from the severed collar until a portion of the cord centre cut by the metal cylinder became visible. This was then cut through. The central part of the cord contained within the cylinder was now driven out a few millimetres by pressure from a metal rod. The end of the protruding cord was cut off and the rest driven out entirely, the other end of the cord also being removed. The remaining rim of cord tissue was inspected to make sure that the cutter had not penetrated the contaminated pial covering; in order to establish this the entire rim of cord tissue was placed in formalin, sectioned serially, and examined microscopically (Fig. 6). Alternatively the

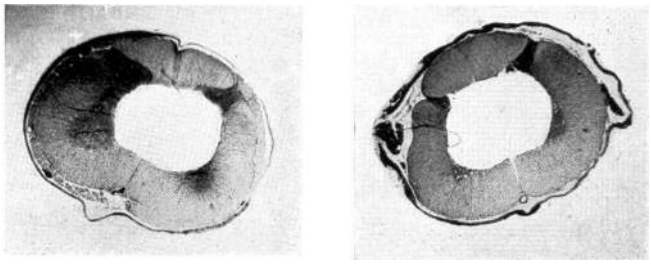

FIG. 6

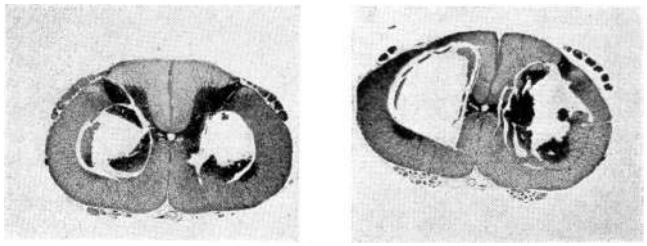

FIG. 7

FIGS. 6 AND 7.—Sections of the remaining cord tissue after removal of the deeper portions (6) with a cylindrical cutter, (7) with an iris knife. In all sections the peripheral portions are intact.

rim was bisected transversely and the upper portion assayed and the lower sectioned as above.

The central rim of the cord was then weighed, broken down in lithium hydroxide, and radioassayed. The cervical cord was assayed in a similar manner.

In some of the sections it was found that the tip of the anterior median fissure was included in the removed cord centre. It is improbable that DBP entered the fissure in any significant amount since it was not outlined in the autographs; nevertheless in order to eliminate this, after the previously described precautions had been taken, a small portion of each lateral white column of the spinal cord was removed with an iris knife and assayed, sections being made of the peripheral rim as before. Examples of such sections are shown in Fig. 7. It will be seen that the anterior median fissure has not been encroached upon. The results of assay were comparable with those of former experiments.

Results

The results (Table VI) show that the blood concentration of drug is high enough to be easily assayed in five minutes after subarachnoid injection. There is a possibility, therefore, that the tissue concentrations obtained are due to the blood in their capillary beds rather than to DBP which has directly penetrated the cord. Studies have already been made of the tissue/blood ratios in respect of the cord after intravenous administration of the drug. The observed results of the cord centre concentration are similarly expressed, and it will be seen from Table VI that the ratios calculated for this series of experiments are far higher for both the cervical and the lumbar cord centres than the corresponding ratios after intravenous injection. DBP has entered the cord by some route other than the blood stream, presumably through the pial membrane in the case of the lumbar cord.

The results recorded for the cervical cord are of special interest, for here it can be seen that the

TABLE VI

DISTRIBUTION OF DIBROMOPROCAINE HYDROCHLORIDE WITHIN THE INTRATHECAL NEURAL ELEMENTS

All values expressed as μg./g. or μg./c.c. unless otherwise stated

Time (min.)	5	10	20	20	25	25
Dose (g.)	0.012	0.0033	0.0033	0.0033	0.0033	0.012
Roots	1,000	320	246	355	63.7	400
C.S.F. lumbar	3,900	1,340	106	190	273	203
Cord periphery lumbar	520	138	66	138	30.9	300
Cord centre lumbar	120	5.3	2.6	5.8	18	49
Cord periphery cervical	17	0.5	0.42	0.7	6.5	2.3
Cord centre cervical	7	0.7	2	0.8	0.5	1.9
C.S.F. cervical	3	±00*	±00*	±00*	5.4	0.9
Blood level	2.9	0.646	0.692	0.78	0.877	2.7
Cord centre lumbar *Ct/Ca*	41.4	8.21	3.76	7.44	20.5	18.1
Cord centre cervical *Ct/Ca*	2.41	1.08	2.39	1.03	0.57	0.705

* ±00 = Too small to estimate. *Ct/Ca* cord ascertained in previous experiment (Tables III and IV) always less than 0.2.

concentration in the cord periphery is higher than that in the C.S.F. which bathes it. There are three possible explanations for this. Either the drug has ascended from the lumbar regions *via* the pial plexus of veins, or by the neural elements within the cord, or the cervical cord has specifically absorbed and concentrated the drug locally from the C.S.F. Specific concentration is unlikely in that it is not a feature of the behaviour of the cord in contradistinction to the roots (Table III). Spread within the cord is likely, since Brierley and Field (1948) have shown that phosphorus injected into the sciatic nerve can be found in the upper reaches of the spinal cord. This passage upwards in the deeper parts of the cord is probably not within the deep veins, since these drain transversely into a superficial longitudinal venous plexus (Mettler, 1942) and do not travel the length of the cord within its substance. It is the results obtained for the cervical cord centres that make some degree of cord penetration by a spinal anaesthetic reasonably certain.

It will be seen that tissue/blood ratios for cord centre and cord periphery have been compared with tissue/blood ratios established for the entire cord. It is possible that tissue/blood ratios for cord centre and cord periphery differ. In order to elucidate this a monkey received an intravenous dose of DBP and the ratios were ascertained for both the central and peripheral parts of the spinal cord. They proved to be 0.21 and 0.23 respectively.

It must be emphasized that it is not possible to compare results obtained from different animals since cord diameters vary and the cutter did not. Consequently a central core removed from a cord of small diameter will encroach further upon the periphery than a similar core removed from a cord of large diameter, and it is in these peripheral portions that a higher concentration of anaesthetic is to be expected.

Note on fixation

Neural fixation has been stated to be responsible for the decrement of drug concentration in the C.S.F.; further, it has been claimed (Stout, 1929) that such fixation prevents ascent of the drug to the vital centres. This is untenable. It has never been suggested that sodium, phosphorus, or bromine suffer such fixation; none the less, compounds of these elements do not appear within the cephalic reaches of the spinal subarachnoid space in any significant amount after intrathecal injection. Further, the rapid appearance of these substances in the venous system clearly points to a more likely mechanism (Howarth and Cooper, 1949).

Of the neural elements within the theca only the spinal roots show regularly a capacity to concentrate a spinal anaesthetic to a level above that in the C.S.F.; to this phenomenon alone is the term "fixation" applied with precision.

"Fixation" implies retention; though this may occur, the total quantity of drug involved must be small since there is no appreciable arrest of the drug within the theca, the anaesthetic appearing almost at once in the venous drainage of the part. In the decrement curves there is no striking difference between those of DBP, sodium, phosphorus, and bromine, and there is no reason to believe that any of the latter three substances is retained within the theca (Howarth and Cooper, 1949). Even in the roots the total amount of DBP retained must be small.

The problem of neural block by the anaesthetic within the cord

In the last section it was shown that DBP could penetrate the spinal cord, and it was of interest to discover whether the concentration there was adequate to block the pathways within the cord. The respiratory pathway was selected for study. According to Starling's textbook (1936) this pathway conveying impulses from the respiratory centres to the cells of origin of the intercostal nerves is situated in the deeper portions of the lateral columns of the cord. Pitts (1940) localized this pathway in the anterior and antero-lateral columns of the cord. Accordingly it was decided to apply an anaesthetic to the cord in an attempt to block this pathway.

Method

In all 45 cats and four monkeys were examined. Laminectomy was performed on the anaesthetized animal extending from the 6th cervical to the 2nd or 3rd thoracic vertebra. Bleeding was controlled and the dura incised along the midline between the caudal and cephalic extremities of the wound. The dural flaps were removed until only a small band of the membrane remained on the ventral aspect of the cord. A swab moistened with warm Dale's Ringer was placed in the wound and a cannula was inserted into the femoral artery and connected to a blood-pressure apparatus. Tambours were placed on the middle of the thoracic wall and upon the antero-lateral abdominal parietes so that thoracic and abdominal respirations could be recorded simultaneously.

The animal was placed upon a board with its caudal end elevated through an angle of 60°, the animal's

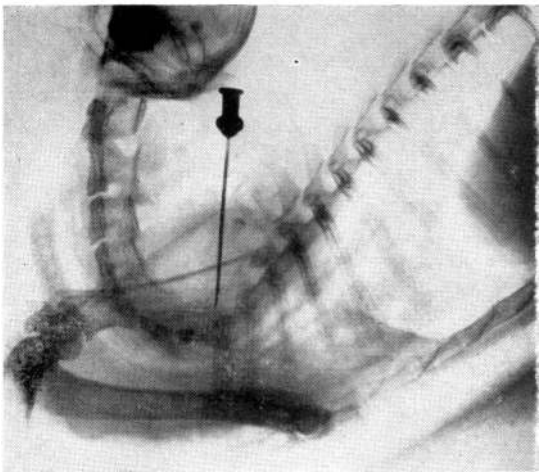

FIG. 8.—Skeletal relationships before application of anaesthetic to cervical cord. Needle indicates the middle of a three-segment laminectomy.

head raised by means of a clamp fixed to its lower jaw, and the extension of the neck continued to the limit of its free movement.

Thus the lower cervical part of the cord was placed in the depths of the wound at the lowest part of the U (Fig. 8). In this way spread of the anaesthetic placed in the base of the U was reduced, sparing the phrenic roots proximally and the intercostal nerves distally.

The smoked drum was set in motion and after a short interval a pledget of wool bearing a warm solution of anaesthetic was placed on the cord and replaced by a further pledget in five minutes. This was repeated throughout the experiment, care being taken to ensure that the anaesthetic had access to the entire periphery of the cord.

Results

The results were consistent and no interruption of the tract was observed after the application of DBP, procaine, cinchocaine, amethocaine, or cocaine itself. Fig. 9 shows the end of such a trace after application of amethocaine followed by percaine.

Before a satisfactory interpretation could be attempted it was essential to discover if a spinal anaesthetic could produce block of the pathways in the cord even if it were placed in contact with them. Thus a small volume of dilute procaine was injected directly into the cord with a fine needle, after first making a small incision in the pia. Under these conditions block of the respiratory pathway was produced with 0.2 c.c. of a 0.5 per cent (w/v) procaine solution (Fig. 10). Neither saline nor water produced such an effect. In Fig. 11 the phrenic roots were paralysed after cinchocaine at T1 by lowering the head, and there was a compensatory increase in thoracic respiration. Later the respiratory centre was paralysed and all respiration ceased. Lowering the thorax did not regularly produce intercostal paralysis, and it was necessary to inject the drug under the free edge of the dura into the thoracic subarachnoid space.

Venous drainage of the spinal subarachnoid space

Various routes whereby a spinal anaesthetic may leave the subarachnoid space have been considered, but none has shown itself in an unequivocal manner to be pre-eminent. Since the appearance of the drug in the blood stream is almost immediate (Howarth and Cooper, 1949) it seemed that a direct venous drainage was the most likely route of departure for a substance administered intrathecally. Further studies have in a large measure confirmed this belief (Howarth and Cooper, 1949). It has

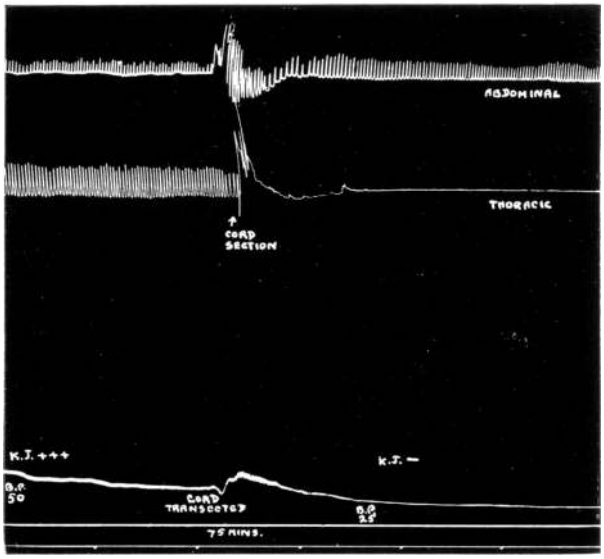

FIG. 9.—Monkey ♂ 7.5 kg. Nembutal, 40 mg./kg. I.V. 1 per cent amethocaine was first applied to the cervical cord. After 50 min. 5 per cent nupercaine was substituted. 75 min. after the original application the respiratory pathway was still conducting. Section of the cord at the level of application produced cessation of thoracic respiration and abdominal increase. The knee-jerks were obliterated. The fall of blood pressure is of doubtful significance; the author has observed such a fall without the application of any anaesthetic with the animal placed in the neck-extended position.

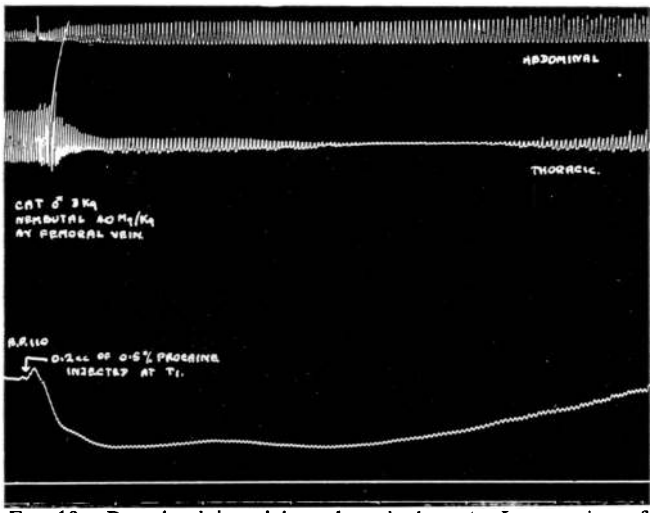

FIG. 10.—Procaine injected into the spinal cord. Interruption of descending respiratory pathway with 0.5 per cent procaine. A similar result may be obtained with a much less volume of procaine (0.05 c.c. of 2 per cent).

been established that the azygos vein is an important channel for the venous drainage of the spinal theca in the cat not only for DBP but also for compounds of sodium, phosphorus, and bromine.

DISCUSSION

From the investigations here recorded the following conclusions appear justifiable.

After introduction into the spinal theca, the concentrations of DBP, sodium, phosphorus, and bromine show a rapid reduction owing to their departure by certain channels. The substances were found in the blood stream as soon as the injection was completed (Howarth and Cooper, 1949).

As regards the anaesthetic, some is absorbed by the spinal roots which usually contain a higher concentration than that present in the C.S.F. The spinal cord too is penetrated by the anaesthetic, though the concentration within it is relatively small and is inadequate to cause a functional transection of the cord. It must not be assumed that this anaesthetic occupies the perivascular spaces, for, although Weed (1914, 1923) has shown that these spaces are in direct communication with the general subarachnoid space, it is probable that the direction of fluid flow is centrifugal rather than centripetal (Weed, 1914). Furthermore, from King's work (1939) it appears that dyes gaining access to the cord from the subarachnoid space do so by a general permeation of the pial membrane rather than by local perivascular ingress. The specific concentration in the roots supports the contention of Tuffier and Hallion (1900) that the site of action of a spinal anaesthetic is centred upon the nerve roots, a conclusion supported by Pitres and Abadie (1901). Smith and Porter (1915) assumed that the pathways of the spinal cord are interrupted, though Babcock (1925) reverted to the earlier view in accordance with the opinion of Labat (1923). Babcock did imply that the cord was slightly affected, but Graffagnino (1926–7) denied even this degree of involvement, a view endorsed by Campbell (1926), Schutz (1928), and Evans (1929). Subsequently, with the notable exception of Sebrechts (1934), there appeared a reversal of opinion by the majority of workers. Thus Koster and Kasman (1929), Russell (1929), Ferguson and North (1932), Grodinsky and Baker (1933), and Vehrs (1934) concluded that the cord is in fact penetrated by the drug. Hill and Macdonald in 1935 stated that spinal anaesthesia was generally accepted to be a root anaesthesia, but Henderson (1937) still

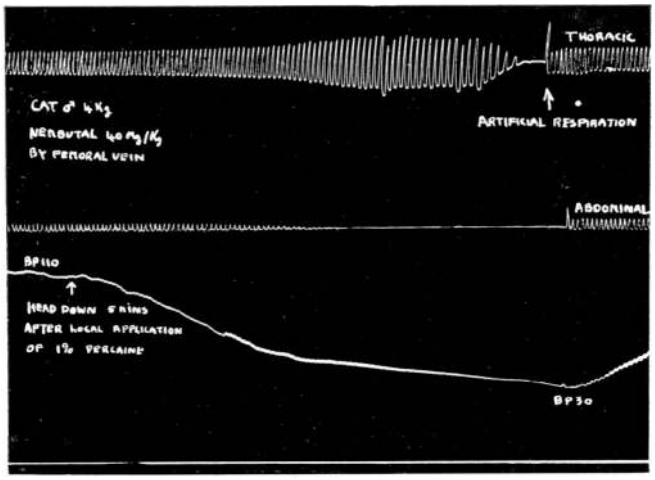

FIG. 11.—Local application 1 per cent cinchocaine to cervical cord. Head down after five minutes. Fall of B.P.; obliteration of abdominal respiration (phrenic roots): compensatory thoracic increase followed by respiratory paralysis, probably owing to arrested activity of respiratory centres. Artificial respiration, B.P. commences to rise.

adhered to anaesthetic cord block. Romberger (1941, 1943) restored the *status quo ante*, holding that the pia mater denies the drug access to the cord. This confusion seems to have arisen from the assumption that an anaesthetic present in the cord must necessarily interrupt the tracts therein. So far as the author is aware a quantitative assay of the drug within the cord has not been reported previously, and it appears that only Harrison and Frank (1932) and Kunlin (1945) have blocked the cord by direct injections of anaesthetic.

It was evident that no study of the causes of cord sequelae of spinal anaesthesia could be undertaken when the basic facts were so ill-understood. The author does not suggest, however, that some of the peripheral tracts are not blocked during spinal anaesthesia, and the fine fibres of the spinothalamic pathway may be interrupted, though he has been unable to devise a satisfactory method for the study of this phenomenon.

Some of the drug appears to ascend within the cord to the cervical regions, a route which has been suggested by Brierley and Field (1948) for phosphorus, and some may pass upwards *via* the pial venous plexus; little passes down the sciatic nerves.

A small quantity leaves the subarachnoid space *via* the lymphatic drainage, thus confirming the presence of the channel suggested by Field and Brierley (1948a and b) in their work with indian ink.

From the blood stream the drug is distributed about the body tissues, but only kidney and liver are able to concentrate it above the blood level, the kidney being far more important than the liver in this respect. The tissue/blood concentrations do not appear to show any significant variations with large changes in blood level and the time interval before autopsy. It would thus appear that tissue retention does not occur except possibly in the 20–30-hour range.

The urine provides the main pathway of removal from the body, and the concentration of the anaesthetic or its end-products is much in excess of the corresponding blood levels, a fact elicited by Burgen and Keele (1948). The blood levels themselves are consistently low. The bile forms a subsidiary excretion channel, and, though some of the products may be reabsorbed by the gut, some appear in the faeces. Some part of the drug containing benzene nuclei persists for considerable periods in the blood, and blood DBP can pass the choroid plexus. Of all the channels draining the spinal subarachnoid space, the venous route appears to be the most important (Howarth and Cooper, 1949). This is not clear in contemporary teaching.

Summary

1. A radioactive spinal anaesthetic dibromoprocaine (DBP) hydrochloride has been prepared.

2. The spinal anaesthetic properties of this drug have been investigated.

3. The distribution of the drug about the body fluids after its intrathecal injection has been studied. It has been shown that the concentration in the spinal subarachnoid space rapidly declines, associated with a rapid rise in urine concentration. The blood level remains persistently low.

4. The tissue distribution of DBP after both intrathecal and intravenous injections has been studied. After intrathecal injections only the spinal roots regularly show any capacity to concentrate the drug above the level existing in the cerebrospinal fluid at the site of injection. Of the tissues examined only kidney (and urine), liver (and bile) appear able to concentrate DBP above the circulating blood concentration. Large variations in time and dose did not produce large changes in the tissue/blood ratios.

5. It has been shown that DBP enters the spinal cord during spinal anaesthesia, though it is improbable that it is able to produce a functional cord transection.

6. Various routes of departure of this anaesthetic from the spinal theca have been studied, and, of these, that furnished by the venous system appears in the cat to be the most important.

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