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STUDIES ON THE BLOOD-BRAIN BARRIER
I. THE BASIS OF DOSAGE FOR ANIMALS OF VARIOUS WEIGHTS

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According to common belief, the cerebral blood-vessels are more permeable in the young than in the adult animal. If any difference in permeability were such that the young blood-vessels allowed the passage of substances normally held back completely in older animals (e.g., many acid dyes), comparatively simple experiments would demonstrate it. If, however, the difference were merely one of degree, it would be apparent only if a satisfactory basis were reached for adjusting, to animals of very dissimilar body-weight, the dosage of a dyestuff or drug intended to pass these blood-vessels and reveal its presence in the nervous tissues by staining, by pharmacological effects, or by other means. None of our colleagues could state by what computation the adjustment should be made, and Clark (1937), discussing the dosage of drugs in general, wrote " there is no satisfactory method of relating dosage to body-weight when the latter varies extensively." The purpose of the present work was to inquire whether any general rule of dosing could be established for causing effects of equal intensity in the nervous tissues of animals of wholly dissimilar weight (and hence age).

Should dosage be related to the weight of the brain ? As Table I shows, the weight of the brain relative to the body-weight is enormously greater in the young than in the adult mouse. To a somewhat lesser extent, the same is true of the rabbit. Although, other things being equal, the concentration of drug in an organ is likely to depend on the concentration in the blood and not on the size of the organ, a consideration of the possible variables involved suggested that these were so numerous that it was advisable to settle the matter by direct experiment.

We had carried out preliminary experiments in which the dosage of dyestuffs and drugs was related to the body-weights or brain-weights of mice of different ages, or was calculated according to the metabolic-rate formula (metabolic rate $\propto$ body-weight $^{2/3}$). The results showed that computation of the dose according to brain-weight invariably led to much more severe effects in young animals. Between the other computations, the results did not permit of final choice; of 7 dyes and 5 convulsant drugs, some appeared to affect mice of different ages more uniformly when administered on the basis of body-weight, others when given according to the metabolic-rate formula.

In the experiments described in the present paper, we adopted statistical methods of assess-

TABLE I

BODY-WEIGHTS AND BRAIN-WEIGHTS IN MICE AT DIFFERENT AGES

	Number examined	Average body-weight	Limits	Average brain-weight*	Limits	Brain-weight as % of body-weight
Old mice	90	41.0 g.	35.5–45.0 g.	0.44 g.	0.41–0.47 g.	1.1
Young adult mice ..	174	20.0 g.	19.0–21.0 g.	0.42 g.	0.40–0.42 g.	2.1
Three-week-old mice ..	174	11.8 g.	10.0–14.0 g.	0.40 g.	0.39–0.42 g.	3.4
Two-week-old mice ..	84	5.6 g.	not recorded	0.36 g.	not recorded	6.4

* Excluding olfactory bulbs and spinal cord.

ment. Because of the very variable response afforded by different animals, and of subjective errors in assessing results, dyes seemed unsuitable for the purpose. We used, therefore, three convulsant drugs (cocaine, strychnine, and picrotoxin) and compared the dosage-mortality curves for mice of three different age-groups; we chose these particular convulsants partly because they are believed to act at quite different levels of the central nervous system, and partly because they have been used previously in experimental work on epilepsy and the permeability of the blood-brain barrier (Cobb, Cohen, and Ney, 1938; Aird, 1939; Aird and Strait, 1944). In other tests we estimated chemically the amount of sulphanilamide passing into the brains of similar animals.

METHODS

Mice were selected according to weight. The weight of a mouse is a fairly precise indication of its age only if conditions of maintenance have been standardized and, at any rate in younger mice, the sizes of the litters have been reasonably constant. For the present purpose we used only mice from litters of two or three animals. These were raised from the age of two weeks under conditions as nearly standardized as possible with the prevailing standards of labour carefully supervised. Under these circumstances, the relation between weight and age was approximately as follows:

12 g.—2½ weeks; 20 g.—5–6 weeks; 35 g.—4 months.

Before experiment, all mice were kept at a constant temperature (±5° F.) for at least three days. On the day of experiment we checked each cage for the presence of surplus food and water. The drugs were injected intravenously, for mice of 20 g. (±0.5 g.),* dissolved in 0.1 c.c. distilled water (0.2 c.c. with sulphanilamide) and introduced in exactly 10 sec. (20 sec. with sulphanilamide) timed with a stop-clock. For mice of the other weights used (12 g. ±0.5 g. and 35 g. ±1.0 g.) the volume of the inoculum was adjusted to the body-weight; the time occupied in injection remained the same.

* In this laboratory mice of 20 g. are used in most experiments and are taken as the standard when comparison between various age-groups is made.

TABLE II

MORTALITIES IN GROUPS OF 10 MICE INJECTED INTRAVENOUSLY WITH VARIOUS CONVULSANT DRUGS

For mice of 20 g. the drugs were dissolved in 0.1 c.c. distilled water and injected in exactly 10 sec. For mice of 12 g. and 35 g. the volume was adjusted to the body-weight; the time of injection remained the same.

Drug	12 g. mice					20 g. mice					35 g. mice				
	Dose μg.	Mortalities Expt. No.				Dose μg.	Mortalities Expt. No.				Dose μg.	Mortalities Expt. No.			
		1	2	3	4		1	2	3	4		1	2	3	4
Strychnine hydrochloride	4	0				7	0				12	0	0	0	0
	5	0	1	1	1	8	0	0	1	1	15	1	0	2	1
	6	3	5	3	3	9	1	0			18	1	2	2	2
	7	3	6	5	5	10	2	3	5	3	21	4	5	5	5
	8	7	8	7	7	11	5				24	10	9	9	9
	10		9	9	9	12		5	7	6					
						14		7	8	8					
						16			10	10					
Cocaine hydrochloride	150	0				200	0				300	2	1	1	
	200	0	0	0		300	0	0	1		400	2	3	3	
	250		2	2		400	2	2	2		500	4	5	5	
	300	5	5	4		500	5	6	5		600	7	6	6	
	350		6	6		600	9	9	9		700	7	8	8	
	400	9	8	8		700		10	10		900		10	10	
	450		10	10											
	500	10													
Picrotoxin	25		0	0		50		0	0		50	0	0	0	
	30	2	3	2		60	3	2	3		70	4	1	1	
	40	4	5	5		70	6	6	5		90		2	2	
	50	4	8	7		80	9	8	8		100	8			
	60	7	10	9		90	9	9	9		110		6	6	
	70	9	10	9		100	10		10		130	10	10	9	
											160	10			

TABLE III

Probit-log. dose relationships for mortalities in mice from convulsant drugs: LD50 values, average slopes, and their standard errors.

Drug	Weight of mice		
	12 g.	20 g.	35 g.
Strychnine slope	8.41 ± 1.23	9.56 ± 1.47	11.80 ± 1.68
LD50 in mg.	0.0069 ± 0.0002	0.0113 ± 0.0003	0.0203 ± 0.0005
Cocaine slope	9.62 ± 1.46	10.22 ± 1.58	5.38 ± 0.85
LD50 in mg.	0.314 ± 0.010	0.472 ± 0.016	0.508 ± 0.024
Picrotoxin slope	6.27 ± 0.90	12.00 ± 1.71	9.00 ± 1.30
LD50 in mg.	0.0419 ± 0.0016	0.0680 ± 0.0017	0.0891 ± 0.0039

In the experiments with convulsant drugs, groups each of 10 mice received doses selected to cover as nearly as possible the whole range of mortalities from 0 to 100 per cent, with at least three values between the limits. We repeated this procedure three or four times on different days, with any variations of dosage suggested by previous experiments.

In the experiments with sulphanilamide, groups each of 3 mice received the drug and were killed 20 min. later. The choice of the time of autopsy was determined by experiments to be reported later. We estimated the amount of sulphanilamide in plasma and brain by the method of Rose and Bevan (1944). The experiment was repeated at approximately weekly intervals.

Results

(a) Convulsant drugs

Table II presents the experimental data for the convulsant drugs used.

The usual method of graphical representation of such data is to express the percentage mortalities as probits and plot these against the logarithm of the dose (Finney, 1947). A statistical analysis of the data of Table II shows that on this scale the dosage-mortality curves for all drugs are linear. The slope of a particular line is thus a measure of the individual variation of the mice to the drug in question (Finney). Further analysis of the data shows that, for each age-group and each drug, the slope of the line does not differ significantly in repeat experiments, indicating that individual variation remained the same throughout the period of experiment—i.e., for at least several days and in more than one batch of mice. Finally, the LD50 values did not differ significantly from one experiment to another. This is not a necessary condition of a satisfactory experiment, but it

TABLE IV

LD50 values and their standard errors expressed in terms of dose per 20 g. mice (mg./mouse) on body-weight, metabolic-rate, and brain-weight formulae.

Basis of assessment of dose	Drug	Weight of mice		
		12 g.	20 g.	35 g.
Body-weight mg./20 g.	Strychnine	0.0115 ± 0.0003	0.0113 ± 0.0003	0.0116 ± 0.0003
	Cocaine	0.523 ± 0.017	0.472 ± 0.016	0.290 ± 0.014
	Picrotoxin	0.0698 ± 0.0027	0.0680 ± 0.0017	0.0509 ± 0.0022
Metabolic-rate formula	Strychnine	0.0097 ± 0.0003	0.0113 ± 0.0003	0.0139 ± 0.0003
	Cocaine	0.442 ± 0.014	0.472 ± 0.016	0.348 ± 0.016
	Picrotoxin	0.0590 ± 0.0022	0.0680 ± 0.0017	0.0610 ± 0.0027
Brain-weight	Strychnine	0.0072 ± 0.0002	0.0113 ± 0.0003	0.0194 ± 0.0005
	Cocaine	0.330 ± 0.011	0.472 ± 0.016	0.485 ± 0.023
	Picrotoxin	0.0440 ± 0.0017	0.0680 ± 0.0017	0.0850 ± 0.0037

This table is derived from Table III. Doses administered according to the metabolic-rate formula were in the following ratio: 20 g. mice 1.00, 35 g. mice 1.46, and 12 g. mice 0.71. The approximate mean brain-weights derived from Table I were respectively 0.42 g., 0.44 g., and 0.40 g. These values and the body-weights were used in calculating the above data. Thus for 12 g. mice the LD50 for cocaine is 0.314. Expressed as dose per 20 g. mice on body-weight basis, this becomes $0.314 \times 20/12 = 0.523$. Calculated on the metabolic-rate formula, the dose would be $0.314 \times 1/0.71 = 0.442$, etc.

enables all the information for each drug contained in the experiments to be combined and represented by the average LD50, the average slope, and their standard errors (Finney).

By doing so we obtained the figures shown in Table III.

Interesting observations can be made from the figures given for the slopes. With strychnine the slopes do not differ significantly for the three age-groups; this implies that the three age-groups have the same degree of variability in their reactions to strychnine. With cocaine the old mice have a significantly lower slope than the other two age-groups, indicating that so far as their reaction to this drug is concerned the old mice vary amongst themselves more than do mice of the other age-groups. For picrotoxin the reverse is true; the young mice vary more amongst themselves than do the other two age-groups. Thus, comparing young and old mice, one may be more variable than the other with respect to one drug and less variable with respect to another. The 20 g. mice show up best for uniformity because with none of the drugs tested is either of the other age-groups significantly less variable.

In Table IV the LD50 is expressed in terms of the three methods of assessing dosage.

For strychnine the LD50 values based on body-weight are almost identical for the three age-groups. For this drug the best basis for calculating the dose is clearly by body-weight. For the other two drugs, none of the bases is completely satisfactory. There is reasonable agreement with picrotoxin for metabolic rate, but the body-weight basis is better when comparing young and 20 g. mice. With cocaine the only reasonable agreement is for brain-weights when comparing old and normal, and for metabolic rate when comparing normal and young mice. For the other group in both instances alternative computation would result in gross error.

All these conclusions are based on the LD50. Since the slopes are not always the same for different age-groups, the above comparative results only apply for the LD50. Different comparative results would be obtained for other levels of mortality, but the conclusion will hold substantially for levels between 20 and 80 per cent.

(b) Sulphanilamide

Table V shows the concentrations of sulphanilamide in the brains of mice of different weights. Obviously, to some extent, the amount of drug in the brain must depend on that in the plasma, and

TABLE V

AMOUNTS OF SULPHANILAMIDE PRESENT IN THE BRAINS OF YOUNG AND OLD MICE INJECTED INTRAVENOUSLY

The first line for each dose refers to one experiment, the second to another, and the third to the last experiment.
Doses for 12 g. mice (1) 0.6 mg. (2) 1.3 mg. (3) 2.0 mg.
„ „ 20 g. „ (1) 1.0 mg. (2) 2.0 mg. (3) 3.0 mg.
„ „ 35 g. „ (1) 2.0 mg. (2) 3.5 mg. (3) 5.0 mg.
For 20 g. mice the above doses were contained in a volume of 0.2 c.c. injected in exactly 20 seconds. For mice of other weights the volume was adjusted to the body-weight, the time of injection remained the same.
Ratio = concentration in brain/concentration in plasma.
Amounts are given in mg. per 100 ml. plasma or 100 g. brain; each pair of entries represent the concentration of sulphanilamide in the plasma and brain of a single mouse.

Dose	Weight of mice								
	12 g.			20 g.			35 g.		
	Plasma	Brain	Ratio	Plasma	Brain	Ratio	Plasma	Brain	Ratio
1	3.54	3.41		3.98	3.03		4.56	2.92	
	3.75	3.10		4.17	2.97		4.75	3.47	
	3.77	3.75		3.15	2.47		4.54	3.35	
	Mean 3.69	3.42	0.93	3.77	2.82	0.75	4.62	3.25	0.70
2	6.27	4.91		5.61	4.83		8.47	5.62	
	5.53	4.92		8.38	5.91		8.20	5.74	
	8.14	5.10		7.37	5.43		6.84	6.16	
	Mean 6.65	4.98	0.74	7.12	5.39	0.76	7.84	5.84	0.74
3	11.88	9.86		14.07	11.67		12.43	8.14	
	15.32	10.88		13.98	8.06		10.84	7.66	
	12.91	9.45		10.92	7.77		11.44	7.69	
	Mean 13.37	10.06	0.75	12.99	9.17	0.71	11.57	7.83	0.68

the ratio between these two quantities would appear to be the more significant figure. These ratios are fairly constant except for the lowest dose in young mice. The last may well represent a testing error resulting in an abnormally high value for the concentration in the brain; it is not, however, sufficiently different from the other figures to warrant rejection of the result. The geometric mean of all nine ratios is 0.75, which may be taken as the ratio, 20 minutes after dosing, for a wide range of doses and weight of mouse. The reason for taking the geometric and not the arithmetic mean will be apparent in the following analysis.

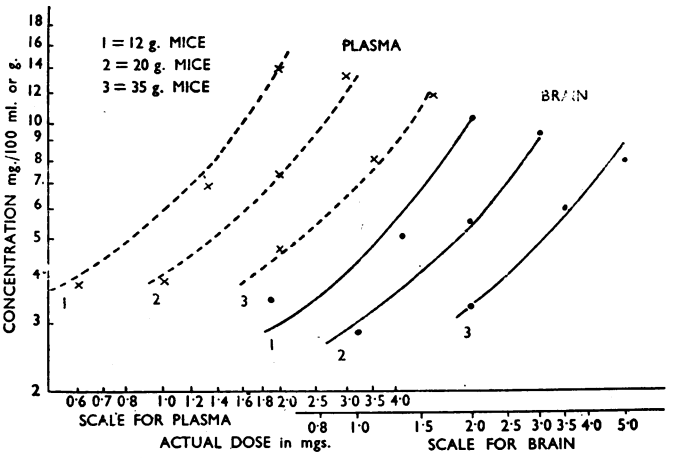

FIG. 1.—Relation between dose of sulphanilamide and concentration in plasma and brain for young, normal, and old mice.

Analysis of results

The mean concentrations in Table V have been plotted on log. scales in Fig. 1—i.e., log. concentration against log. dose. Some curvature is apparent in each graph, but it is clear that all curves are substantially parallel and that the relation between the plasma curves for young, normal, and old mice is similar to that for the brain curves. If the curves are in fact parallel and occupy relatively the same position within both sets (subject of course to experimental error), then the interpretation is greatly simplified. If the curves were not parallel, no uniform basis of calculating the dose would be possible, because the basis would then depend on the magnitude of the dose. If, for instance, the curves converged for low doses (and diverged for high), the ratios of the doses for young, normal, and old mice giving the same concentration of drug in the brain would be closer together for low than for high doses. The assumption that the curves are parallel was made, therefore, as a working hypothesis, to be subject to a rigorous statistical test at a later stage. If this assumption could be justified, a uniform basis for assessing doses would exist; this basis would be the ratio of the doses which yield the same concentration of drug in the brain (or plasma) for all three age-groups.

The assumption is embodied by representing the six curves by the equations in Table VI.

Fitting these lines to the log. of the values of Table V by the method of Least Squares, we obtain the following values for the constants: $m_1=0.169$, $m_2=0.421$, $a=0.726$, $A=0.602$, $b=0.909$, $c=1.233$. These refer to the origin -0.222 for plasma and brain, and the theoretical curves are plotted in Fig. 1; it will be seen that the fit is reasonably good. The next step is to assess whether this fit is consistent with the experimental error. To do this we calculate for each animal the quantities

Y=log. concentration in plasma + log. concentration in brain.

Z=log. concentration in plasma − log. concentration in brain.

The variation within the groups of three mice for each dose and age group represents the experimental error for these quantities. An analysis of variance of Z shows that no significant variation arises between groups. It follows that the ratio of concentration in the brain to the concentration in the plasma may be assumed constant for all doses and age-groups of mice examined.

The fitted lines for the quantities Y for the three age-groups are derived by summing the corre-

TABLE VI

Sulphanilamide in the brains of mice: formulation of equations for log. concentration of sulphanilamide *v.* log. dose

	Plasma	Brain
Young	$y=a+bx+cx^2$	$y=A+bx+cx^2$
Normal	$y=a+b(x-m_1)+c(x-m_1)^2$	$y=A+b(x-m_1)+c(x-m_1)^2$
Old	$y=a+b(x-m_2)+c(x-m_2)^2$	$y=A+b(x-m_2)+c(x-m_2)^2$

sponding equations for the plasma and brain. The variance of the nine group means about these lines is 0.0065 based on 4 degrees of freedom. This has to be compared with the experimental error variance. We calculate that the variance of Y within groups of three mice is 0.0093 based on 18 degrees of freedom, accordingly the error variance of the group means is $0.0093/3=0.0031$. The variance about the fitted lines is not significantly greater than this. The fit of the lines is, therefore, adequate and the working assumptions justified.

We have now shown that within the experimental error, the ratio of the concentration in the brain and plasma is the same for all doses and weight of animal within the range examined, and also that the curves connecting the concentration in the plasma (and brain) with log. dose for the three age-groups are parallel.

The effect of age (or weight) of mouse on the concentration in the plasma and brain is therefore given entirely by the two constants m_1 and m_2 calculated previously—i.e., $m_1=0.169$, $m_2=0.421$. These are in terms of log. dose ; their antilogs. are 1.476 and 2.637. The relation between the doses to give the same concentration for young, normal, and old mice is, therefore, 1.00 to 1.476 to 2.637 respectively. Expressed as normal=100, we get 67 : 100 : 178.

In Table VII we compare these ratios with the three bases for assessing dose.

The ratios found agree almost entirely with the body-weight basis of assessment. The experimental error in the ratios found is unlikely ($p=0.05$) to exceed ± 12%. We can therefore confidently exclude the other two bases of assessment of the dose, since the experiment is on a sufficient scale to discriminate conclusively between the three bases and thus gives a clear-cut result. Fewer animals would have been insufficient and a larger number unnecessary. The conclusion is that the correct basis of assessment of dose is by body-weight.

TABLE VII

Sulphanilamide in the brains of mice: ratio of dose per mouse calculated in various ways compared with doses causing equal concentrations of the drug in the brain.

Basis of assessment of dose	Weight of mice		
	12 g.	20 g.	35 g.
Found experimentally ..	62	100	169
Body-weight	67	100	178
Brain-weight	95	100	105
Metabolic-rate formula ..	72	100	146

An estimate of the constant brain/plasma concentration may be obtained from a and A calculated above. The difference $A-a=1.876$ which corresponds to an antilog. of 0.75. This, of course, is identical with the geometric average determined previously from Table III, and is the justification for using the geometric in place of the arithmetic mean of the ratios.

Summary and Conclusions

The foregoing experiments sought to determine whether there exists any uniform basis of dosage, for mice of different weights, of drugs penetrating the blood-brain barrier. As an indication of their presence in the nervous tissue, we determined the mortality curves of three convulsant drugs, strychnine, cocaine, and picrotoxin. We also estimated chemically sulphanilamide passing into the brain. All the drugs were given intravenously under rigidly standardized conditions, thereby eliminating factors concerned with absorption from the alimentary canal or from the tissues.

Although, proportionately to the body-weight, the brain is relatively very much heavier in the young animal, dosage on the basis of brain-weight nearly always results in the administration of too large amounts of drug. Apart from this statement, no general rule can be formulated. With strychnine and sulphanilamide, dosage on the basis of body-weight gave results most nearly uniform in the various mice. With picrotoxin, dosage according to the metabolic-rate formula gave reasonably uniform results over the complete range of weights, but dosage on the basis of body-weight gave closer correlation between immature mice and young adults. With cocaine no basis of dosage appeared satisfactory for all weights. Computation according to the metabolic-rate formula yielded greatest uniformity between immature mice and young adults, according to the brain-weight between young adults and old mice.

In the absence of any uniform basis of dosage for mice of widely different weights, the recognition of small differences in the permeability of the cerebral blood-vessels of young and old animals would be a matter of considerable difficulty.

References

Aird, R. B. (1939). *Arch. Neurol. Psychiat.*, **42**, 700.
Aird, R. B., and Strait, L. (1944). *Arch. Neurol. Psychiat.*, **51**, 54.
Clark, A. J. (1937). *Handb. exp. Pharmakol.*, Berlin: Springer.
Cobb, S., Cohen, M. E., and Ney, J. (1938). *Arch. Neurol. Psychiat.*, **40**, 1156.
Finney, D. J. (1947). *Probit Analysis*, Cambridge University Press.
Rose, F. L., and Bevan, H. G. L. (1944). *Biochem. J.*, **38**, 116.