The diminution of the cytocidal effect of DMM by dose-fractionation is similar to that observed with dose-fractionation of radiation.[3-5, 9] The effect is predictable from the nature of the dose-survival curve which in the case of DMM and radiation is characterized by an initial shoulder followed by an exponential decline. With each dose fraction a portion of drug will be "wasted" traversing the shoulder portion of the dose-survival curve, the more prominent the shoulder the greater will be the loss of cytocidal effect by dose-fractionation. In the case of HN2, in which the dose-survival curve is exponential throughout with only a slight shoulder, the cytocidal effect is similar whether the drug is administered as a single dose or divided into several fractions.

The reduction in cytocidal effect of DMM on dose-fractionation can also be explained by repair of sub-lethal damage inflicted by each dose fraction and such cumulative repair exceeds that observed following single dose therapy. This study supplies further evidence that mechanisms exist to repair DMM-induced cell damage but fails to reveal evidence for analagous repair processes following HN2 therapy.

The possibility that division delay is more marked following single dose than fractionated dose DMM therapy has not been entirely excluded but this interpretation of the results is considered unlikely for the reasons stated above (see discussion on limitations of extrapolation method, and Fig. 3).

If this explanation was valid then it would also follow that division delay following single and fractionated dose HN2 therapy was identical.

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REFERENCES

1. M. M. ELKIND, *Jap. J. Genet. suppl.* **40**, 176 (1964).
2. G. J. GOLDENBERG and P. ALEXANDER, *Can. Res.* **25**, 1401 (1965).
3. M. M. ELKIND and H. SUTTON, *Nature, Lond.* **184**, 1293 (1959).
4. M. M. ELKIND and H. SUTTON, *Radiat Res.* **13**, 556 (1960).
5. M. M. ELKIND, H. SUTTON-GILBERT, W. B. MOSES, T. ALESCIO and R. W. SWAIN, *Radiat. Res.* **25**, 359 (1965).
6. M. M. ELKIND, H. SUTTON-GILBERT, W. B. MOSES and C. KAMPER, *Nature, Lond.* **214**, 1088 (1967).
7. P. ALEXANDER and Z. B. MIKULSKI, *Biochem. Pharmac.* **5**, 275 (1961).
8. W. C. DEWEY, R. M. HUMPHREY and A. CORK, *Int. J. Radiat. Biol.* **6**, 463 (1963).
9. M. M. ELKIND, T. ALESCIO, R. W. SWAIN, W. B. MOSES and H. SUTTON, *Nature, Lond.* **202**, 1190 (1964).

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Hyposensitivity to 5-hydroxytryptamine in the isolated stomach fundus of the newborn rat—I.

Organ preparation and neonatal quantitative behaviour of the hyposensitivity*

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THE PHARMACOLOGICAL experimentation on the newborn rat is lacking in precise quantitative data, if compared with that on the adult animal, and our current state of knowledge is based mostly upon toxicological and teratological studies of reviews by Done,[1] Yeary *et al.*,[2] Yaffe and Back.[3] The data concerning the sensitivity and the responsiveness of newborn animals towards different drugs are particularly scarce (Yaffe and Back[3]). Although it is generally assumed that the animal in the period

* Part of this data was presented at the XIV Congresso Nazionale della Società Italiana di Farmacologia, Trieste, June 5–7, 1967.

shortly after birth is more sensitive than the adult to the drug toxic effects (Nyhan[4]), several Authors have pointed out than *in vivo* this conclusion is not necessarily valid (Done[1], Yeary, Benish *et al.*[2]). Also *in vitro* experiments show that strips of the gastro-intestinal tract of newborn rats often respond to some biological transmitters and certain drugs with less sensitivity than in the adult (Cugurra and Vaccari[5]).

This paper describes the occurrence of a reduced sensitivity to 5-hydroxytryptamine (5-HT) in the gastric fundus of newborn rats.

METHODS

Male and female rats of Wistar albino strain were used; the animals were born and bred in our animal division.

Preparation of the newborn rat gastric fundus

Because of the small size of the stomach of the newborn rat, we employed the whole fundus. The stomach, rapidly removed after animal sacrifice, was immediately put in Tyrode solution (37°); the fundus was then separated (Fig. 1A), and leaving *in situ* the alimentary *bolus* to keep the organ wall stretched, the tissue was cut longitudinally following the lesser curvature, from the esophagus junction till ½ of the greater curvature (Fig. 1B, 1C). The strip so obtained (Fig. 1D, 1E) was kept in Tyrode at 37°, vigorously and constantly oxigenated with pure O_2 in order to avoid differences related to pO_2 variations in the reactions of the isolated smooth muscle preparations towards 5HT (Smith and Vane[6]).

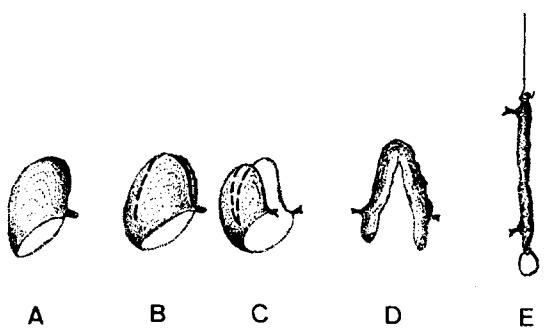

FIG. 1. Diagram showing the method of preparing the isolated fundus from a newborn rat stomach. In the figure, the fundus after dissection.

Preparation of the adult rat fundal strips

The adult rat gastric fundus was cut according to Offermeier:[7] the method, that used only a portion of the fundus, was similar to that used by us for the newborn, and allows a good comparison of the results. The fundal preparations so obtained were connected to a lightly loaded and practically isotonic lever; the magnification by the lever was about 1:30. The drugs were assayed according to the procedures of cumulative dose-response curves, with the sequence ½ log 10 or log 10 (Ariëns and De Groot,[8] Van Rossum and Van den Brink,[9] Van Rossum[10]). Doses are expressed as base.

Statistical analysis

The results obtained from the cumulative curves were evaluated according to Ariëns, Van Rossum *et al.*[10] The statistical significance of the differences and the omoschedasticity were assessed on the mean values of pD_2 (affinity) and α (intrinsic activity) by the Snedecor's F-ratio (Snedecor[11]). The calculations were made with an Electronic Computer IBM 7040.

RESULTS AND DISCUSSION

Figure 2 shows cumulative dose-response curves obtained from preparations of newborn rats at various age-days until the typical adult values are reached. Table 1 gives the variation of the receptorial affinity to the 5HT at several days of neonatal period. Mean sensitivity at the 1st day postpartum is lower by a factor of 23 in comparison with that of the adult; also extreme values of about

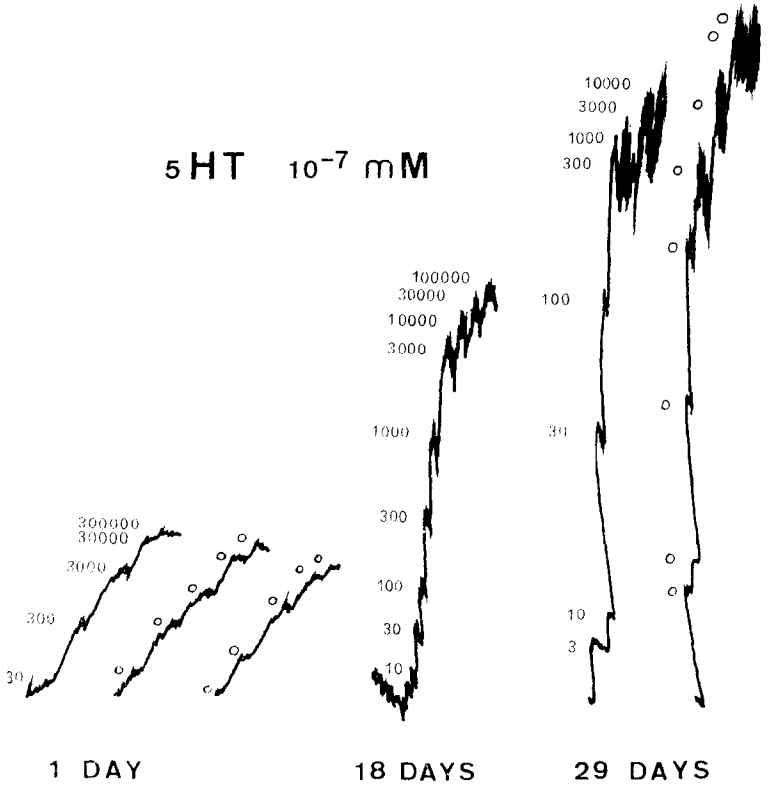

FIG. 2. Cumulative dose-response curves of 5-HT on isolated fundal preparations of newborn rats at 1, 18 and 29 age-days: in the first 18 days the same doses give lesser responses than on the 29th day (adult response).

TABLE 1. NEONATAL AFFINITY (pD_2) AND RELATIVE HYPOSENSITIVITY TOWARDS 5-HT AT VARIOUS AGE DAYS

Age	pD_2* ± S.E.	Est/org†	Significance‡	Omoschedasticity	Relative hyposensitivity§
Adult	7·96 (±0·21)	47/27	—	—	1·0
1 day	6·60 (±0·44)	35/19	$P < 0.01$	negative	−23·0
3 days	7·15 (±0·32)	39/18	$P < 0.01$	positive	−6·3
6 days	7·60 (±0·33)	29/14	$P < 0.1$	positive	−2·3
9 days	7·83 (±0·21)	50/22	n.s.	positive	1·0
18 days	7·38 (±0·29)	23/12	$P < 0.01$	positive	−4·7
29 days	7·95 (±0·29)	9/4	n.s.	positive	1·0

* pD_2 (affinity): negative logarithm of the concentration of agonist required to produce 50 per cent of the maximal response obtained in the system.

† Est/org.: number of cumulative dose-response curves/number of organs used.

‡ Significance levels of the neonatal affinity in comparison to that of the adult.

§ Relative hyposensitivity expressed as factor of the affinity in the adult animal (= 1·0; inverse log. scale).

230 times less have been run. This great variability is shown in the control of the omoschedasticity, negative for the data from 1-day-old rats in spite of the numerous experiments done. This implies the concurrence of several experimental factors, as the lever-loading, the oxygenation, the difficulty of cutting the strips, which are all important factors in such preparations, beside the specific reasons for the hyposensitivity. The pD_2 of the newborn, till the 6th day, differ from the adult. Within 18 to 29 days post-partum a complete normalization of the values is reached. To check if hyposensitivity in the gastric fundus of the newborn rat also applies to drugs acting on different receptor systems, we have tested on the same preparation a typical cholinergic drug, the furtrethonium (HFur) (Fig. 3).

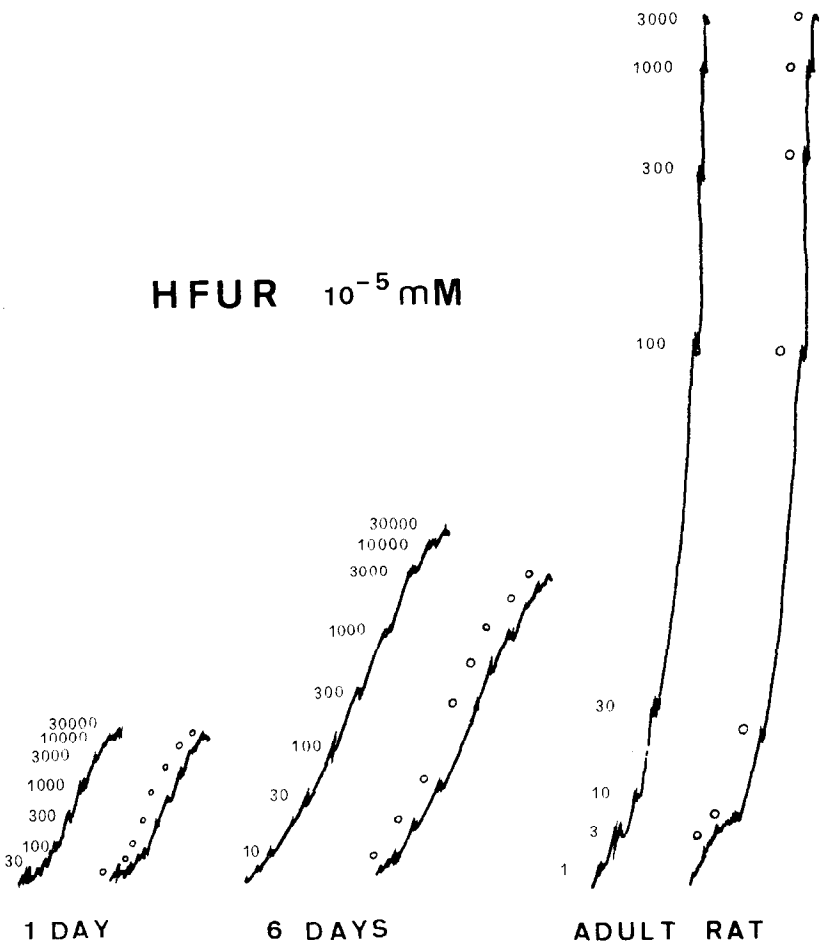

FIG. 3. Cumulative dose-response curves of the cholinergic furtrethonium (HFur) on isolated stomach fundus preparations of newborn (1, 6 age-days) and adult rats: a mild hyposensitivity occurs in the first 6 days post-partum.

Also for this compound, a weak neonatal hyposensitivity till the 6th age-day occurs. This hyposensitivity, never exceeded the factor 6 *in minus* (Table 2).

From the present results, it is possible to conclude that the hyposensitivity in the newborn rat to 5-HT is partially due, besides the aforementioned concurrence of experimental factors which are difficult to control, to an intrinsic minor contractility of the smooth muscle not yet fully developed.

TABLE 2. NEONATAL AFFINITY (pD_2) AND RELATIVE HYPOSENSITIVITY TOWARDS FURTRETHONIUM AT VARIOUS AGE DAYS

Age	pD_2* ± S.E.	Est/org.†	Significance‡	Relative hyposensitivity§
Adult	6·49 (±0·06)	62/26	—	1·0
1 day	5·72 (±0·20)	32/14	$P < 0·01$	−5·8
6 days	5·79 (±0·20)	12/5	$P < 0·01$	−5·0
9 days	6·28 (±0·22)	21/9	$P < 0·05$	−1·6
12 days	6·28 (±0·18)	21/9	$P < 0·05$	−1·6
18 days	6·43 (±0·21)	8/4	n.s.	1·0

* pD_2 (affinity): negative logarithm of the concentration of agonist required to produce 50 per cent of the maximal response obtained in the system.

† Est/org.: number of cumulative dose-response curves/number of organs used.

‡ Significance levels of the neonatal affinity in comparison to that of the adult.

§ Relative hyposensitivity expressed as factor of the affinity in the adult animal (= 1·0; inverse log. scale).

This is shown by the experiments done with HFur, drug acting on receptor systems other than that of serotonin and exhibiting a certain degree of minor affinity in the newborn rat. But the more important reason of this hyposensitivity must lie in other causes, located probably at level of the receptor system for the 5-HT (sialic acids?) and of the receptor-5HT interaction, as indicated by preliminary data.

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REFERENCES

1. A. K. DONE, *Clin. Pharmac. Ther.* **5**, 433 (1964).
2. R. A. YEARY, R. A. BENISH and M. FINKELSTEIN, *J. Pediat.* **69**, 663 (1966).
3. S. J. YAFFE and N. BACK, *Pediat. Clin. N. Am.* **13**, 528 (1966).
4. W. L. NYHAN, *J. Pediat.* **59**, 1 (1961).
5. F. CUGURRA and A. VACCARI, *Minerva Pediat.* **19**, 731 (1967).
6. D. J. SMITH and J. R. VANE, *J. Physiol., Lond.* **186**, 284 (1966).
7. J. OFFERMEIER, *Serotonin and its derivatives, a study on structure-activity relations*, Thesis, 45. Thoben Offset, Nijmegen (1965).
8. E. J. ARIËNS and W. M. DE GROOT, *Archs int. Pharmacodyn. Thér.* **99**, 193 (1954).
9. J. M. VAN ROSSUM and F. G. VAN DEN BRINK, *Archs int. Pharmacodyn. Thér.* **143**, 240 (1963).
10. J. M. VAN ROSSUM, *Archs int. Pharmacodyn. Thér.* **143**, 299 (1963).
11. G. W. SNEDECOR, *Statistical Methods*, 5th edn. Iowa State University Press, Ames, Iowa (1956).

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Hydroxyindole-*O*-methyltransferase in several avian species

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THE MAMMALIAN pineal gland has the capacity to *O*-methylate *N*-acetylserotonin to form the gonad-inhibiting compound, melatonin.[1] The formation of melatonin is catalyzed by an enzyme, hydroxyindole-*O*-methyltransferase (HIOMT), which is uniquely localized in the mammalian pineal gland.[2] The enzyme was found to be present in the chicken[3] and in other animal species.[4] This report describes some properties of HIOMT in the Japanese quail (*Coturnix coturnix japonica*) and in other avian species that are different from the mammalian enzyme.