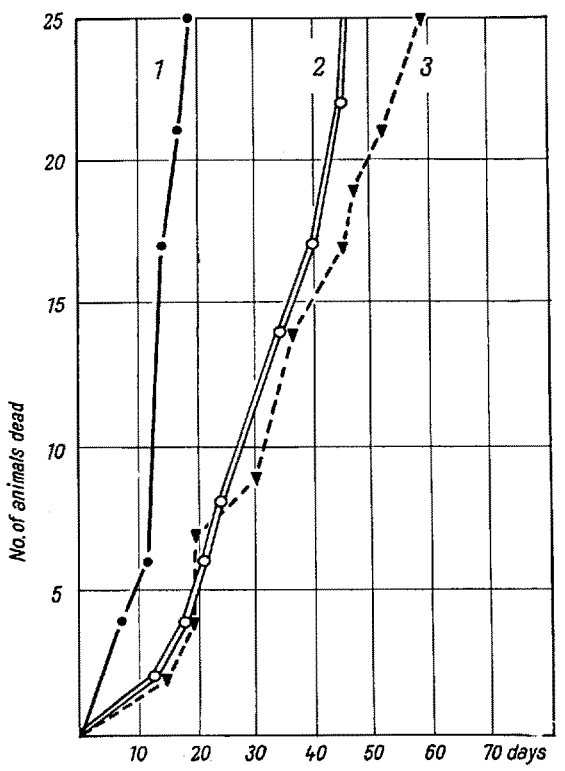
these tumor cells considerably; mean survival was increased from about 12 to about 30 and 35 days respectively, as can be seen from a representative experiment in the Figure. (Three such experiments were carried out.) The dose schedule was as follows: H_2O_2 360 mg/kg in 1 cm³ water daily for seven days; urea peroxide 800 mg/kg in 1 cm³ water daily for two days followed by 400 mg/kg daily for four days. Hydrogen peroxide has already been tested by CHORAŽY *et al.*[4] and SUGUIRA[5] and found to have no effect on the Ehrlich ascites tumor cells; however, CHORAŽY *et al.* administered it in drinking water, whereas the dose used by SUGUIRA (150 mg/kg) was far lower than ours.



Survival of mice inoculated with Ehrlich ascites tumor cells: (*1*) no treatment, (*2*) treatment with H_2O_2, (*3*) with urea peroxide

Most of the mice treated with these compounds were autopsied; interestingly, all showed solid tumors. Four specimens of these specimens, kindly examined by Dr. G. H. FRIEDELL (Massachusetts Memorial Hospital), showed essentially identical findings. One report reads as follows: 'Compatible with Ehrlich ascites tumor. There are extensive areas of necrosis. Stroma is minimal in amount. Tumor is infiltrating muscle and shows perineural and perivascular lymphatic invasion.'

The following compounds did not prolong survival as the treated animals showed well developed ascites tumors similar to the controls: Hydroxyheptyl peroxide (Lucidol, Buffalo, New York, 20 mg/kg in 0·05 cm³ ethanol daily for two days), *p*-menthane hydroxperoxide (Lucidol, 400 mg/kg in 0·5 cm³ mineral oil daily for three days), dicumyl peroxide (G. L. Cabot, Cambridge, Massachusetts, 1000 mg/kg in 0·5 cm³ mineral oil once), succinic acid peroxide (Lucidol, 80 mg/kg for five days in 1 cm³ water).

We are presently engaged in an extensive study of the effect of peroxide compounds of different stability on various experimental tumors.

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Zusammenfassung

Die Lebensdauer von mit Ehrlich-Ascites-Karzinom injizierten Mäusen wird durch Wasserstoffsuperoxyd und Harnstoffperoxyd auffallend verlängert. Der Tumor wird dabei von der Ascites- in die solide Form umgewandelt.

[4] M. CHORAŽY, A. GETTLICH, L. GÓRAL, B. KOLOCZEK, E. MOLAWKA, B. PENAR, and Z. SZWEDA, Nature *182*, 394 (1958).

[5] K. SUGUIRA, Ann. N. Y. Acad. Sci. *76*, 575 (1958).

An Antagonism Between 3-4 Dioxyphenylalanine (DOPA) and 5-Hydroxytryptophan (5-HTP)

Recently, it has been suggested that 5-hydroxytryptamine and norepinephrine may be mediators at the C.N.S. level. In connection with a previous theory of HESS[1], BRODIE[2] suggested that the two opposing subcortical systems, integrating autonomic somatic and psychic functions, might be regulated by serotonin (trophotropic system) and norepinephrine (ergotropic system). There is also some evidence that an antagonism between serotonin and norepinephrine occurs both *in vitro*[3–5] and *in vivo*[3,6].

Table I

Treatment	No. of animals	Sleeping time (min)	*P*-value
(1) Hexobarbital (H)	20	33 ± 2·9	
(2) 5-HTP + H. . .	20	54 ± 3·4	(1)–(2) 0·001 > *P*
			(4)–(2) 0·001 > *P*
(3) DOPA + H. . .	20	32 ± 3·5	(1)–(3) N. S.
			(4)–(3) N. S.
(4) 5-HTP + DOPA + H.	20	34 ± 3·3	(1)–(4) N. S.

5-HTP 50 mg/kg and DOPA 50 mg/kg were injected intraperitoneally 30 min before the injection of hexobarbital 80 mg/kg i. p. Room temperature (18–19° C)

The purpose of the experiments reported here was to investigate whether or not norepinephrine could antagonize the potentiation of barbiturate narcosis induced by

[1] W. R. HESS, *Das Zwischenhirn* (Benno Schwabe and Co., Basle 1954).

[2] B. B. BRODIE and P. A. SHORE, Ann. N. Y. Acad. Sci. *66*, 631 (1957).

[3] R. JAQUES, H. J. BEIN, and R. MEIER, Helv. physiol. Acta *14*, 269 (1956).

[4] S. GARATTINI and L. VALZELLI, Boll. Soc. ital. Biol. sper. *32*, 288 (1956).

[5] S. GARATTINI and L. VALZELLI, Boll. Soc. ital. Biol. sper. *32*, 292 (1956).

[6] P. GORDON, F. J. NADDY, and M. A. LIPTON, Science *128*, 531 (1958).

serotonin[5,7,8]. Since serotonin and norepinephrine penetrate the blood-brain barrier with difficulty, their precursors, DL-5-hydroxy-tryptophan (5-HTP, California Biochemical Research) and DL-2,4-dioxyphenylalanine (DOPA-Hoffmann-La Roche), were used.

Table II

Treatment	No. of animal	Sleeping time (min)	*P*-value
(1) Pentobarbital P.	18	33 ± 2·9	
(2) 5-HTP + P. .	18	50 ± 3·7	(1)–(2) $0{\cdot}01 > P > 0{\cdot}001$
			(4)–(2) $0{\cdot}05 > P > 0{\cdot}02$
(3) DOPA + P. .	18	36 ± 2·5	(1)–(3) N. S.
			(4)–(3) N. S.
(4) 5-HTP + DOPA + P.	18	39 ± 3·0	(1)–(4) N. S.

5-HTP 50 mg/kg and DOPA 20 mg/kg were injected intraperitoneally 30 min before the injection of pentobarbital 50 mg/kg. Room temperature (22–23° C)

Female swiss mice of the average weight of 20 g were injected i. p. with 5-HTP or DOPA (dissolved in distilled water) 30 min before the administration of pentobarbital (50 mg/kg i. p.) or hexobarbital (80 mg/kg i. p.). The sleeping time was determined, by observing the duration of the loss of righting reflex.

The results obtained are summarized in Tables I and II.

It was observed that 5-HTP potentiates both pentobarbital and hexobarbital. On the contrary DOPA, at the concentration tested, did not affect the barbiturate sleeping time, but completely reversed the potentiation induced by 5-HTP. These results do not necessarily imply an antagonism between norepinephrine and serotonin. Recent data showed that DOPA and 5-HTP compete for the decarboxylase enzyme(s)[9]. Assuming that serotonin is responsible for the barbiturate increased activity, the observed antagonism could be interpretated as a decrease in the formation of serotonin in the presence of DOPA. On the other hand, if serotonin potentiates barbiturate action by a peripheral effect, the antagonism observed with DOPA could be explained on the basis of the known antagonism between serotonin and norepinephrine[10].

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Riassunto

Recentemente è stata formulata l'ipotesi che la 5-idrossitriptamina e la noradrenalina agiscano come mediatori a livello del S.N.C. I risultati ottenuti dall'A. dimostrano che la diossifenilalanina può antagonizzare il potenziamento della narcosi barbiturica indotto dal 5-idrossitriptafano.

[7] P. FORNAROLI and M. KOLLER, Il Farmaco, ed. sci. *9*, 546 (1954).

[8] F. N. FASTIER, Exper. *12*, 351 (1956).

[9] A. YUWIBER, E. GELLER, and S. EDUSON, Arch. Biochem. Biophys. *80*, 162 (1958).

[10] It should be also recalled that a potentiation between serotonin and norepinephrine has been observed [11].

[11] R. MEIER, T. TRIPOD, and E. WIRZ, Arch. int. Pharmacodyn *109*, 55 (1957).

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Immunological Unresponsiveness in Chickens Induced by Human γ-Globulin

Many authors[1] have reported the supression of the immune response in fetal and new-born rabbits due to excessive amounts of foreign protein antigens injected into animals. Recently, WOLFE *et al.*[2] succeeded in producing immunological unresponsiveness in newly hatched chickens by injecting them with bovine serum albumin. The present report deals with the antibody production in chickens following injections of human γ-globulin into embryos and infant animals.

Rhode Island Red embryos and chicks were used in the experiments. Chicken embryos of Group I were intravenously injected on the 12th day of incubation with 16 mg of human γ-globulin (HGG), followed by a rest until the sixth week of age. Infant chicks of Group II received subcutaneously eight injections of HGG (16 mg/injection). First injection was given one day after hatching and other injections in four-day intervals until the 32nd day of postnatal life. Group III of uninjected chicks served as control. When the experimental animals of all groups were 42 days old, they were inoculated with 40 mg of HGG. Control bleedings were performed in the sixth week of age prior to the injection of 40 mg of HGG, and the test bleedings seven days after HGG had been administred. All sera were tested on the same day to avoid variation in titers[3]. The animals of the Group II and Group III were further treated with HGG (40 mg/injection). The animals were hatched and maintained in our laboratory.

Anti-human globulin antibody in chicken sera was determined by Coombs anti-human globulin technique. Red cells from a single donor of group A, cDE/cDE were used. A 5-% suspension was prepared in 10 ml of an anti-D+E serum diluted 1:50. The same antiserum and the same dilution was used throughout the experiment. The incubation was 60 min at 37°C. The sensitized cells were washed three times in saline and a 2-% suspension was prepared. The chicken sera were made up in serial doubling dilutions and distributed in 0·05 volume in tubes (7 × 45 mm). To each tube 0·05 ml of sensitized red cell suspension was added and allowed to stand for 2 h at 37°C. The results were read microscopically on the slide. Parallel series of diluted chicken sera were set up for the determination of heteroagglutinins. Nonsensitized A, cDE/cDE red cells were used as antigen.

The Table records the results obtained with chicken sera after the injection of 40 mg of HGG. Control bleeding sera of all three groups of chicks, obtained prior to the injection of 40 mg of HGG, were also tested, using both sensitized and nonsensitized red cells. But they have shown only the activity of hetero-agglutinins and not the activity of anti-human globulin antibody.

These results indicate that chickens which received several injections of HGG in the early period of postnatal life produced antibody at a much lower level, when assayed on the 7th day after the challenging injection. In the 13th week, the antibody production reached the level of the control group, tested in the seventh week of age.

[1] R. HANAN and J. OYAMA, J. Immunol. *73*, 49 (1954). – F. J. DIXON and P. H. MAURER, J. exp. Med. *101*, 245 (1955). – B. CINADER and J. M. DUBERT, Brit. J. exp. Path. *36*, 515 (1955). – R. T. SMITH and R. A. BRIDGES, Transpl. Bull. *3*, 145 (1956).

[2] H. R. WOLFE, C. TEMPELIS, A. MUELLER, and S. REIBEL, J. Immunol. *79*, 147 (1957). – C. H. TEMPELIS, H. R. WOLFE, and A. MUELLER, Brit. J. exp. Path. *39*, 323, 328 (1958).

[3] N. GENGOZIAN and H. R. WOLFE, J. Immunol. *78*, 401 (1957)