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)

Preliminary treatment	No. of chick	Age, 6 weeks	Age, 7 weeks		Age, 13 weeks	
			Range	Mean ± SE	Range	Mean ± SE
Group I Single i.v. injection of 16 mg HGG (12-days embryos)	10	One injection of 40 mg HGG	64–512	217,6 ± 50,25		
Group II Eight s. c. injection of 16 mg of HGG each at age 1–32 days	10	One injection of 40 mg HGG	16–64	40 ± 6,54	*64–512	234 ± 52,7
Group III None (control)	12	One injection of 40 mg HGG	64–512	213,3 ± 45,86	128–1024	298 ± 72,33

* Nine chicks tested

A single injection of antigen given to 12-days old embryos had no effect on the latter immune response. This data seems to support the view of SIMONSEN[4] that the day of injection may be of significance for reduction of immunological responsiveness.

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Zusammenfassung

Wiederholte Injektionen von menschlichem γ-Globulin in der ersten Nachgeburtsperiode, führte bei Kücken zu immunologischer Toleranz gegenüber diesem Antigen.

[4] M. SIMONSEN, Acta path. microbiol. scand. *39*, 21 (1956).

Cholinesterase in Human Salivary Glands

In case of tumour in or near the salivary glands, surgical removal of the tumour and surrounding glandular tissue is the standard treatment. Advantage of this fact was taken to obtain fresh human salivary gland tissue for estimation of cholinesterase activity. Previously, the cat submaxillary and parotid gland (STRÖMBLAD[1]) have been found to contain cholinesterase.

Methods. Only apparently healthy tissue was used; the tumour was cut off with a wide margin. The tissue was carefully cleaned, washed in saline, weighed, minced with scissors, and ground in a glass homogenizer with Krebs' bicarbonate-Ringer. The volume in ml was then brought up to five times the weight of the tissue in g.

The cholinesterase estimations were made manometrically using Warburg flasks of conventional shape and size. The main compartment contained 0·2 ml of the homogenate and 1·5 ml of Krebs' bicarbonate-Ringer solution. The substrates, in the side bulb in 0·3 ml, were acetylcholine, methacholine, or benzoylcholine in concentrations giving final reaction molarities of 0·011, 0·035, and 0·290, respectively. The substrates were added after 15 min of temperature equilibration. The gas was N_2 with 5% CO_2 and the temperature 37° C. Readings were taken every 5 min for 40 min. In all experiments, a thermobarometer and enzyme blank were used. Corrections were made for changes in these flasks and for non-enzymic hydrolysis. All estimations were made in duplicate. The activity was expressed in μl CO_2 evolved / 30 min / g of tissue.

In some experiments, a small volume of eserine salicylate giving a reaction mixture molarity of $3{\cdot}63 \times 10^{-6}$ was tipped into the main chamber 25 min after estimation had begun. The CO_2 evolution was almost completely abolished and this was taken as evidence that the reactions studied were enzymic breakdown of choline esters.

Table

	Acetylcholine	Methacholine	Benzoylcholine
Parotid glands	♂ 1335 ± 97·7 (n = 8)	493 ± 49 (n = 7)	75 ± 233 (n = 6)
	♀ 1035 ± 64·8 (n = 8)	405 ± 30 (n = 5)	70 ± 20·0 (n = 5)
Submaxillary glands	♂ 1463 ± 29·8 (n = 6)	573 ± 28·6 (n = 5)	133 ± 26·9 (n = 5)
	♀ 1183 (n = 3)	388 (n = 3)	50 (n = 3)

Results and Discussion. The human parotid and submaxillary glands showed a moderately high cholinesterase activity (see Table). The activity with acetylcholine and methacholine was much higher than with benzoylcholine, in fact in some cases no enzymic splitting of benzoylcholine was found. The substrate concentration-activity curve showed an almost regular bell-shaped form. Thus the cholinesterase in the human salivary glands is mainly true cholinesterase (acetylcholine esterase). The same is true for cat glands (STRÖMBLAD[1]).

The acetylcholine splitting activity was higher for male parotids than for female parotids when analyzed with Student's t-test ($P < 0{\cdot}05$). When the acetylcholine-splitting activity of male and female glands was plotted against the age of the patients, a correlation was found for females between age and activity ($r = 0{\cdot}807$, $P < 0{\cdot}01$). No such close correlation could be demonstrated for the male parotid glands. Analysis of variance

[1] B. C. R. STRÖMBLAD, Acta physiol. scand. *41*, 118 (1957).