conjugate itself or by β-amanitin released after penetration of the conjugate into the cell[5]. Experiments are in progress to find which concentrations of AMA-BSA would block chromosomal RNA synthesis in macrophages without interfering with RNA synthesis in lymphocytes. The use of AMA-BSA at these concentrations may help to elucidate the role of macrophage RNA synthesis in antibody production carried out *in vitro* by mixed cultures of macrophages and lymphocytes.

We thank Mr L. Franchi for technical assistance. This investigation was supported by grants from CNR, Rome.

G. BARBANTI-BRODANO
L. FIUME

Istituto di Microbiologia and
Istituto di Patologia Generale,
Università di Bologna,
Via San Giacomo 12 *and* 14,
40126 *Bologna*

Received January 23; revised March 5, 1973.

1 Fiume, L., and Wieland, Th., *FEBS Lett.*, **8**, 1 (1970).
2 Wieland, Th., and Wieland, O., in *Microbial Toxins* (edit. by Kadis, S., Ciegler, A., and Ajl, S. J.), **8**, 249 (Academic Press, New York, 1972).
3 Fiume, L., in *Pathology of Transcription and Translation* (edit. by Farber, E.), 105 (M. Dekker, New York, 1972).
4 Fiume, L., Campadelli-Fiume, G., and Wieland, Th., *Nature New Biology*, **230**, 219 (1971).
5 Derenzini, M., Fiume, L., Marimozzi, V., Mattioli, A., Montanaro, L., and Sperti, S., *Lab. Invest.* (in the press).
6 Virolainen, M., *J. Exp. Med.*, **127**, 943 (1968).
7 Koprowski, H., and Fernandes, M. V., *J. Exp. Med.*, **116**, 467 (1962).
8 Mage, M. G., Evans, W. H., and Peterson, E. A., *Proc. Soc. Exp. Biol. Med.*, **127**, 478 (1968).
9 Miller, R. G., and Phillips, R. A., *Proc. Soc. Exp. Biol. Med.*, **135**, 63 (1970).
10 Raidt, D. J., Mishell, R. I., and Dutton, R. W., *J. Exp. Med.*, **128**, 681 (1972).
11 Haskill, J. S., *J. Exp. Med.*, **130**, 877 (1969).
12 Unanue, E. R., *Nature*, **218**, 36 (1968).
13 Feldmann, M., and Palmer, J., *Immunology*, **21**, 685 (1971).
14 Wagner, H., Feldmann, M., Boyle, W., and Schrader, J. W., *J. Exp. Med.*, **136**, 331 (1972).
15 Plotz, P. H., and Talal, N., *J. Immunol.*, **99**, 1236 (1967).
16 Shortman, K., Williams, N., Jackson, H., Russel, P., Byrt, P., and Diener, E., *J. Cell Biol.*, **48**, 566 (1971).
17 Hoskins, J. M., *Virological Procedures* (Butterworths, London, 1967).

Mode of Action of Hallucinogenic Molecules

5-HYDROXYTRYPTAMINE (5-HT) has been implicated as a neurotransmitter[1–3] and may play a key role in the functional organization of the central nervous system of higher animals. As hallucinogenic compounds such as lysergic acid diethylamide (LSD) and mescaline have many structural similarities to 5-HT (refs. 4, 5), the mode of action of these compounds may be related in some manner to their effects on 5-HT receptors. For example, LSD applied iontophoretically to the surface of brain neurones either mimics or inhibits the action of 5-HT (refs. 2, 6). Such studies are, however, difficult to interpret because of problems in regulating drug concentrations during iontophoresis, and because of the ill-defined role of 5-HT in brain function.

We have used the salivary glands of the adult blowfly, *Calliphora*, to investigate this proposed relationship between 5-HT and hallucinogenic drugs. In this animal 5-HT acts as a hormone that turns on fluid secretion by the salivary glands[7,8]. These organs are simple tubes comprised of a single cell type; they are not innervated or vascularized and lack a thick connective tissue and musculature[9]. The glands function well *in vitro* and some of the intermediate steps between activation of the receptor by 5-HT and the onset of secretion have been worked out[7,10]. For our discussion it is sufficient to know that stimulation of the gland with 1×10^{-7} M 5-HT results in a rapid increase in fluid secretion from a resting level of 0.5 nl min^{-1} to a stimulated level of 30 nl min^{-1} (Fig. 1). That a hormone-receptor interaction is taking place is indicated by the exquisite sensitivity of the glands to 5-HT (refs. 7, 8) and by the observation that when the hormone is washed off the secretion rate quickly returns to the resting level (Fig. 1). Thus with this simple experimental system it is possible not only to regulate carefully the concentration of drugs in the vicinity of the 5-HT receptor, but also continuously to monitor both the onset and recovery of the secretory response to different drugs.

When LSD is applied to the salivary gland there is a rapid increase in fluid secretion rate, just as when 5-HT is applied (Fig. 1). On the other hand, the recovery after LSD treatment was greatly prolonged. The glands continued to secrete in spite of continuous washing to remove every trace of extracellular LSD. Similar results were obtained with mescaline and 3,4-dimethoxyamphetamine.

The following evidence suggests that LSD interacts with the same tryptaminergic receptor as does 5-HT and is not stimulating fluid secretion by various non-specific mechanisms. (1) LSD can reproduce all the principal physiological and biochemical changes usually observed during the action of 5-HT. For example, LSD causes the transepithelial potential to go rapidly negative as does 5-HT. LSD also duplicates the increase in positivity of the transepithelial potential induced by 5-HT when chloride in the bathing medium is replaced by the impermeant anion isethionate[10]. Finally, LSD can elevate the level of intracellular cyclic AMP in the same way as 5-HT. (2) 5-HT can prevent LSD from acting on the gland. When salivary glands were treated with 10^{-9} M LSD in the presence of 1×10^{-6} M 5-HT for 10 min there was the normal increase in the rate of secretion. When the two agonists were replaced with control medium, however, the secretion rate rapidly returned to the resting level. In the control experiment, glands treated for 10 min with 1×10^{-9} M LSD alone also showed an increase in secretion but when LSD was washed off there was a typical slow recovery. Thus, interaction of LSD with the active sites on the gland can be prevented if the available receptor sites are occupied with 5-HT molecules.

One possible explanation of these results is that the LSD-receptor interaction is readily reversible and that the slow recovery depends solely on the slow dispersion of a pool of molecules accumulated while in contact with the gland. Consequently, when the compound is withdrawn from the bathing medium, the receptor sites may continue to be stimulated by molecules diffusing out from this intracellular reservoir of drug molecules. This is apparently not the case because glands which have been pretreated with LSD are not affected when they are subsequently washed for short periods (up to 10 min) with gramine (1×10^{-4} M) or harmaline (1×10^{-4} M), both of which completely inhibit the action of 5-HT (1×10^{-8} M)

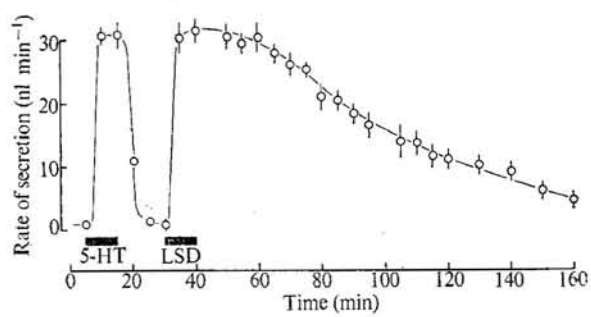

Fig. 1 Effect of 5-HT (10^{-7} M) and LSD (10^{-7} M) applied for 10 min (solid bars) on the rate of secretion of isolated salivary glands. At other times the glands were bathed in control medium which was replaced every 5 min. Each point represents the mean of six glands and the standard errors are shown.

within 5 min. These observations suggest that the receptor is continuously occupied with LSD and is apparently immune to the immediate action of these inhibitory molecules. If treatment with these antagonists was continued for longer than 10 min, however, a slow decrease in secretion developed. Thus, both gramine and harmaline can accelerate the recovery of salivary glands after treatment with LSD.

Our observations indicate that various hallucinogenic drugs detach very slowly from the 5-HT receptor (the property of an irreversible antagonist). The following hypothesis attempts to explain how this observation can be reconciled with their continued ability to stimulate the glands. That part of the hallucinogenic molecule which resembles tryptamine[4,5] may interact with the receptor as does the normal agonist, but, in addition, the remaining parts of the molecule may bind in a non-specific way to regions adjacent to the tryptaminergic receptor. After a successful receptor interaction, that part of the molecule which resembles tryptamine may begin to leave the receptor in the usual reversible manner except that the additional non-specific binding may not reverse so readily. Consequently, the molecule is kept in the vicinity of the receptor with which it can make repeated interactions. The hallucinogenic molecule may flap up and down on the receptor using the additional binding sites as semi-permanent attachment points. When the active part of the molecule leaves the receptor, the latter will be temporarily free to interact with other molecules. Thus, gramine and harmaline may accelerate the release of LSD from the receptor by inserting themselves between LSD and the receptor site when the latter is briefly unoccupied. As is observed experimentally, the effect of such antagonists on the LSD response would be slow, for the incoming molecule must compete with an LSD molecule already poised above the receptor.

The results of this study thus provide a clue as to the possible mechanism by which hallucinogens act at the receptor level. They may mimic the action of 5-HT on the tryptaminergic receptor but their action is greatly prolonged because they fail to disengage properly from the active site.

M. J. BERRIDGE
W. T. PRINCE

ARC Unit of Invertebrate Chemistry and Physiology,
Department of Zoology,
Downing Street,
Cambridge CB2 3EJ

Received February 23, 1973.

1 Bradley, P. B., *Intern. Rev. Neurobiol.*, **11**, 1 (1968).
2 Aghajanian, G. K., *Fedn. Proc.*, **31**, 91 (1972).
3 Gershenfeld, H. M., and Stefani, E., *Adv. Pharmacol.*, **6A**, 369 (1968).
4 Chothia, C., and Pauling, P., *Proc. US Nat. Acad. Sci.*, **63**, 1063 (1969).
5 Kang, S., and Green, J. P., *Proc. US Nat. Acad. Sci.*, **67**, 62 (1970).
6 Boakes, R. J., Bradley, P. B., Briggs, I., and Dray, A., *Brit. J. Pharmacol.*, **40**, 202 (1970).
7 Berridge, M. J., *J. Exp. Biol.*, **53**, 171 (1970).
8 Berridge, M. J., *J. Exp. Biol.*, **56**, 311 (1972).
9 Oschman, J. L., and Berridge, M. J., *Tissue and Cell*, **2**, 281 (1970).
10 Berridge, M. J., and Prince, W. T., *Adv. Insect Physiol.*, **9**, 1 (1972).

Effect of Human Chorionic Somatomammotrophin and Human Chorionic Gonadotrophin on Phytohaemagglutinin-induced Lymphocyte Transformation

THERE is, as yet, no experimental evidence to explain why the foetus, a homograft, is not rejected by the mother[1]. Two of the many interesting features of the implanting blastocyst are that it is surrounded by maternal lymphocyte-like cells, and it has the ability to synthesize large quantities of the glycoprotein human chorionic gonadotrophin (HCG), which can be detected in both blood and urine almost immediately after implantation. The presence of HCG in urine forms the basis of a pregnancy test. At a later stage, the syncytiotrophoblastic cells which have differentiated from the blastocyst synthesize the polypeptide hormone human chorionic somatomammotrophin (HCS)[2]. It has been found in maternal blood during the fourth week after conception. Its concentration increases rapidly throughout pregnancy, reaching its peak in the last trimester[3]. It is possible that either or both of these polypeptide hormones, which are specific to the human placenta, may modify the immunological competence of maternal lymphocytes and could be factors in preventing rejection of the foetus. Using a standard technique, we have investigated the effect of the hormones on phytohaemagglutinin (PHA)-induced transformation of peripheral lymphocytes, which is a measure of cell-mediated immunity.

Heparinized whole blood from a healthy male donor was mixed with Hank's solution containing 6% dextran in 0.9% saline in a (v/v) ratio of 10 to 6. The mixture was allowed to

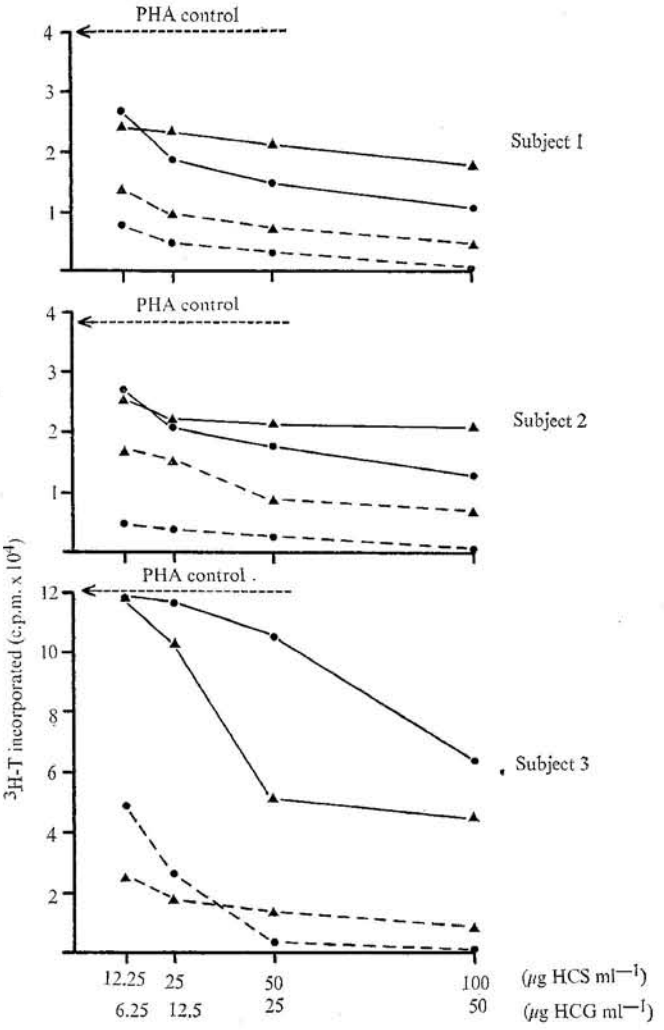

Fig. 1 Dose-response curves of HCS on PHA-induced lymphocytes of three different subjects. Each point on the graph represents the geometric mean of triplicate samples. Arrows refer to the ^{3}H-thymidine incorporated (c.p.m. $\times 10^4$) into lymphocytes by the action of PHA alone. ●---●, HCS added on day 1, PHA on day 2; ▲---▲, HCG added on day 1, PHA on day 2; ●—●, HCS and PHA added simultaneously on day 1; ▲—▲, HCG and PHA added simultaneously on day 2.