as bovine gamma globulin (BGG) and hen's ovomucoid (OM) varied similarly with the H-2 type. Furthermore, a remarkable coincidence exists between the pattern of immune responsiveness of mouse strains to low doses of hapten conjugates of BGG or OM, as presently described, and the responsiveness of the same strains to immunization with a synthetic amino acid polymer, (His, Glu)-Ala-Lys, as described by McDevitt and Chintz (*4*). The immune responsiveness to (His,Glu)-Ala-Lys was shown to be under control of a single gene which is closely linked to the H-2 locus (*4*). Segregation analysis will be necessary to establish whether a similar situation exists in the control of the immune response to low doses of hapten-protein conjugates. If this proves to be the case, it would mean that genetic differences at a single locus may influence the development of immune responses to a wide range of different antigens with, as yet, no obvious structural similarities or antigenic cross reactivity (*9*).

NELSON M. VAZ
BERNARD B. LEVINE

Department of Medicine, New York University School of Medicine, New York 10016

References and Notes

1. M. A. Fink and V. A. Quinn, *J. Immunol.* **70**, 61 (1953); W. F. Barth, C. L. McLaughlin, J. L. Fahey, *ibid.* **95**, 781 (1965); N. L. Warner, N. M. Vaz, Z. Ovary, *Immunology* **14**, 725 (1968); N. M. Vaz and A. Prouvost-Danon, *Progr. Allergy* **13**, 111 (1969).
2. W. R. Sobey, J. M. Magrath, A. H. Reisner, *J. Immunol.* **96**, 511 (1966); D. Hardy and D. Rowley, *Immunology* **14**, 401 (1968); G. Biozzi, C. Stiffel, D. Mouton, *Ann. Inst. Pasteur* **115**, 965 (1968).
3. H. O. McDevitt and M. Sela, *J. Exp. Med.* **122**, 517 (1965); B. B. Levine and B. Benacerraf, *Science* **147**, 517 (1965); P. H. Maurer and P. Pinchuck, *Immunity Cancer and Chemotherapy* (Academic Press, New York, 1967), p. 319.
4. H. O. McDevitt and A. Chinitz, *Science* **163**, 1207 (1969); H. O. McDevitt and B. Benacerraf, *Advan. Immunol.* **11**, 31 (1969).
5. B. B. Levine, M. J. Fellner, V. Levytska, *J. Immunol.* **96**, 707 (1966); B. B. Levine and V. Levytska, *ibid.* **98**, 648 (1967).
6. I. Mota and J. M. Peixoto, *Life Sci.* **5**, 1723 (1966); N. M. Vaz and Z. Ovary, *J. Immunol.* **100**, 169 (1968); B. B. Levine and N. M. Vaz, *Int. Arch. Allergy Appl. Immunol.*, in press.
7. These animals were later challenged with 100 μg of BPO-BGG in alumina gel, and made antibody responses similar to the responses of normal control mice.
8. D. L. Gasser, *J. Immunol.* **103**, 66 (1969).
9. Experiments testing for cross reactivity between BGG and OM conjugates were done and were negative.
10. Supported by contract No. DADA 17-67-7119 from the U.S. Army Medical Research and Development Command. N.M.V. is a visiting scientist from the Instituto Nacional do Cancer, Rio de Janeiro, Brazil. B.B.L. is a career investigator of the Health Research Council of the City of New York. We acknowledge the excellent technical assistance of Henry Chang, Jr. We thank Dr. Victor Nussenzweig, Department of Pathology, New York University School of Medicine, for his stimulating interest in this work.

9 January 1970

Catecholamine Biosynthesis in Brains of Rats Treated with Morphine

Abstract. *In the brains of rats without tolerance to morphine, the accumulation of [^{14}C]dopamine formed from [^{14}C]tyrosine injected intracisternally is increased, reaching a maximum in the hypothalamus and striatum 1 hour after administration of morphine. In tolerant rats, the rate of incorporation of carbon-14 into dopamine and into norepinephrine in these areas is more than twice that in animals that have received only one injection of morphine.*

There is evidence that biogenic amines in the central nervous system interact with morphine and other narcotic analgesic drugs in two ways: morphine alters amounts of amines in the nervous system, and changes in the amounts of amines alter the response to morphine. In most experimental animals, the administration of morphine causes release of dopamine and norepinephrine from the brain in the first hours after the initial injection of the drug (*1*). In animals made tolerant by long-term treatment with morphine, there is no depletion of amine (*2*). However, the rate at which tolerance develops may be changed by simultaneous administration of drugs that alter the amounts of biogenic amines in nervous tissue. Drugs such as reserpine lower amounts of amines; inhibitors of monoamine oxidase increase amounts of amines by inhibiting their catabolism (*3*). The exact relationship between biogenic amines and narcotic drugs may not be defined by measurements of the catecholamine content of nervous tissue since these reflect only gross changes and may not reveal local changes or alteration in the rate of turnover of amines. We have studied the rate of incorporation of ^{14}C from [^{14}C]tyrosine into dopamine and norepinephrine in whole brain and in regions of the brains of tolerant and nontolerant rats.

To measure accumulation of labeled catecholamine, we injected [^{14}C]tyrosine (uniformly labeled) into the fourth ventricle of rats 10 minutes before killing them. The rate of its conversion to [^{14}C]dopa (dihydroxyphenylalanine), and, in succession, to [^{14}C]dopamine and [^{14}C]norepinephrine was measured after the amines were adsorbed from an acidic extract of brain on alumina, and separated on Dowex-50 columns (*4*).

Thirty to 90 minutes after one injection of morphine (60 mg/kg body weight), the rate of accumulation of [^{14}C]dopamine in whole brain was significantly increased over that in controls injected with saline, reaching a maximum 60 minutes after the injection (Fig. 1). The rates of incorporation of ^{14}C into norepinephrine and into dopa were apparently unaltered, except at one time. Calculations of the rate of conversion of tyrosine to dopamine and norepinephrine in brain in vivo are complicated by the fact that

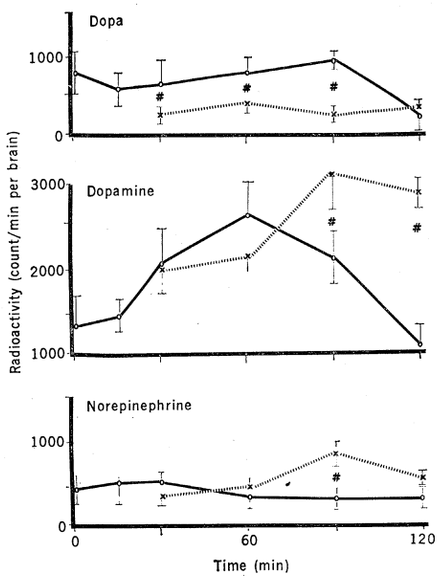

Fig. 1. The conversion of [^{14}C]tyrosine to [^{14}C]dopa, [^{14}C]dopamine, and [^{14}C]norepinephrine in rat brain. Each rat was injected intracisternally with 5 μc of [^{14}C]-tyrosine (uniformly labeled, 370 μc/μmole) and killed 10 minutes later. The brains were homogenized in 0.4*N* perchloric acid, the metabolites were separated on alumina and Dowex-50 columns, and the radioactivity was measured by scintillation spectrometry (*4*). The values for animals injected with saline are shown as zero-time values. All other rats were injected with morphine (60 mg/kg body weight) and killed at times from 15 to 120 minutes later; (———) rats injected once; (- - - - -) rats receiving a tenth daily injection of morphine (dose increasing from 20 to 60 mg/kg). The standard deviations are indicated by the perpendicular lines, and the starred values show significant differences ($P < .001$) between one and ten injections. At an average specific radioactivity of [^{14}C]tyrosine of 5.56 μc/μmole (calculated from time curves of [^{14}C]tyrosine remaining in brain during the 10-minute pulse), 478 count/min per brain (norepinephrine control value) represents a rate of synthesis of norepinephrine of 0.225 nmole/hour per gram of brain.

endogenous pools of amines and dopa alter the specific activity of the precursor, and by widely varying rate constants of the enzymes involved in the metabolic pathway (*5*). The conversion of tyrosine to dopa is presumably rate limiting (*6*), so that [^{14}C]dopa would not be expected to accumulate. Newly synthesized dopamine will accumulate as the end product in neurons containing dopamine and also in those containing norepinephrine since dopamine β-oxidase has a relatively high Michaelis constant for dopamine (*7*). [^{14}C]Norepinephrine found in the brains of treated rats may be the sum of two opposing reactions, an increased synthesis of the amine and an increased depletion by morphine. In addition, it is possible that newly synthesized norepinephrine is preferentially released in response to morphine, as it is upon stimulation of sympathetic nerves (*8*).

To study the accumulation of labeled catecholamines in regions of the brain, we dissected brains into six areas (*9*) after a 10-minute exposure to [^{14}C]tyrosine; the same areas of six brains were combined to provide enough tissue for the isolation of metabolites.

When labeled tyrosine was administered intracisternally endogenous pools of tyrosine in the brain became highly labeled; however, the distribution of the labeled amino acid was uneven, varying from 1510 count/min per milligram of protein in the cerebral cortex to 9970 count/min per milligram of protein in the hypothalamus. Since the distribution of [^{14}C]tyrosine was not uniform, the radioactivity in the metabolites was expressed as the counts per minute per milligram of protein in the area, corrected to a common pool of [^{14}C]tyrosine. Labeling in the areas of brains from control rats was compared with that in areas from rats receiving morphine 1 or 2 hours before being killed (Table 1). There was an increase in [^{14}C]dopamine in the hypothalamus and the striatum 1 hour after the injection. One hour later, the rates of labeling of all the metabolites in these areas were lower than in controls.

In rats made tolerant by increased doses of morphine given over 10 days, as in naive rats, there was an increase in [^{14}C]dopamine formed from [^{14}C]tyrosine. This increase was greater and more prolonged than that after a single injection; the amount was significantly greater than control amounts at all times from 30 to 120 minutes in whole brain (Fig. 1). The significant differences between the first and tenth injection (Fig. 1) include these changes in the accumulation of [^{14}C]dopamine at 90 and 120 minutes after the injection of morphine. In the experiments in which brain areas were examined, the rats were made tolerant by five daily injections of morphine and then killed 2 hours after the fifth injection. In the brains of these animals, the conversion of [^{14}C]tyrosine to all metabolites was higher in the hypothalamus, striatum, and midbrain, than in the same areas from control rats or rats injected once (Table 1). In all areas, the rate of labeling of norepinephrine was higher than in the control or naive animal. Thus, at a time when the rats were tolerant to the analgesic and hypothermic actions of morphine, the neurochemical response of increased amine formation was more prolonged than after the initial injection.

Table 1. Conversion of [^{14}C]tyrosine to [^{14}C]dopa, [^{14}C]dopamine, and [^{14}C]norepinephrine in six areas of rat brain. Rat brains were dissected into six areas (*9*), which were combined from six rats which had been injected with saline (control) or morphine (60 mg/kg) 1 or 2 hours before being killed (M-1 and M-2). Another group received the same dose of morphine for 5 days and was killed 2 hours after the last injection (5 M-2). Each value is the average of two pooled samples from six rats each. The methods are described in Fig. 1. The values are expressed as counts per minute per milligram of protein in tissue area, corrected to 10^6 counts per minute in [^{14}C]tyrosine.

Treatment	Radioactivity					
	Cerebellum	Medulla	Hypothalamus	Striatum	Midbrain	Cortex
			Dopa			
Control	2.75	5.54	43.73	9.24	6.57	0.76
M-1	2.48	5.54	33.71	13.46	9.84	0.55
M-2	2.82	5.64	18.73	2.21	7.53	0.53
5 M-2	4.47	5.56	65.58	41.91	11.93	0.90
			Dopamine			
Control	3.87	14.07	194.02	70.67	42.29	11.41
M-1	3.24	11.14	238.08	93.90	40.19	9.85
M-2	2.67	10.56	130.63	20.39	29.25	6.51
5 M-2	5.57	21.56	209.10	97.10	68.02	15.56
			Norepinephrine			
Control	3.75	5.62	53.65	8.80	10.47	1.79
M-1	4.07	3.49	52.27	5.63	6.48	0.68
M-2	2.97	7.19	36.71	2.38	7.06	1.02
5 M-2	8.04	15.27	176.81	10.02	16.68	2.04

The difference in the effects of the initial and the long-term injections of morphine suggests that in the brains of tolerant rats, the catecholamine biosynthetic pathway operates more effectively than it does in the brains of naive rats. It is interesting that when norepinephrine turnover is measured as the rate of disappearance of intracisternally administered [^{3}H]norepinephrine, the turnover of the amine in the whole brain of tolerant rats is not different from that in controls (*10*). This lack of effect may reflect the success of the biosynthetic pathway in maintaining the amounts of the biogenic amines.

The amine response to morphine treatment is not limited to the accumulation of newly synthesized dopamine and norepinephrine. Accumulation of another biogenic amine, serotonin, is seen in the brains of mice treated with morphine if an inhibitor of monoamine oxidase is used to block the further metabolism of serotonin (*11*). That protein synthesis is involved in the development of tolerance to morphine is suggested by two kinds of evidence. (i) After first being inhibited, protein synthesis is increased above normal levels in the brains of rats treated 24 hours earlier with morphine (*12*), and (ii) inhibitors of protein synthesis retard the development of tolerance to narcotic analgesic drugs (*11, 13*). It is possible that tolerance to many of the pharmacological effects of narcotic drugs is produced by an induction of the enzymes involved in the synthesis of the biogenic amines in the nervous system.

DORIS H. CLOUET
MILTON RATNER
New York State Research Institute for Neurochemistry and Drug Addiction, Ward's Island, New York 10035

References and Notes

1. M. Vogt, *J. Physiol.* (*London*) **123**, 451 (1954); G. P. Quinn and B. B. Brodie, *Med. Exp.* **4**, 349 (1961); E. W. Maynert and G. I. Klingman, *J. Pharmacol. Exp. Therap.* **135**, 285 (1962).
2. L-M. Gunne, *Acta Physiol. Scandinav.* **58**, Suppl. 204, 1 (1963); J. Sloan, J. W. Brooks, A. J. Eisenman, W. R. Martin, *Psychopharmacologia* **3**, 291 (1962).
3. H. Nybäck, Z. Borzecki, G. Sedvall, *Eur. J. Pharmacol.* **4**, 395 (1968); K. J. Rogers and J. A. Thornton, *Brit. J. Pharmacol.* **36**, 470 (1969).
4. A. H. Anton and D. F. Sayre, *J. Pharmacol. Exp. Therap.* **138**, 360 (1962); H. Nybäck and G. Sedvall, *ibid.* **162**, 294 (1968).

5. N. H. Neff, S. H. Ngai, C. T. Wang, E. Costa, *Mol. Pharmacol.* **5**, 90 (1969); G. C. Sedvall, V. K. Weise, I. J. Kopin, *J. Pharmacol. Exp. Therap.* **159**, 274 (1968).
6. M. Levitt, S. Spector, A. Sjoerdsma, S. Udenfriend, *J. Pharmacol. Exp. Therap.* **148**, 1 (1965).
7. S. Udenfriend, in *Biochemistry and Pharmacology of the Basal Ganglia*, E. Costa, L. J. Cote, M. D. Yahr, Eds. (Raven Press, New York, 1966).
8. G. Sedvall, V. K. Weise, I. J. Kopin, *J. Pharmacol. Exp. Therap.* **159**, 274 (1968).
9. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
10. M. J. Neal, *J. Pharm. Pharmacol.* **20**, 950 (1968).
11. E. L. Way, H. H. Loh, F. H. Shen, *Science* **162**, 1290 (1968).
12. D. H. Clouet, in *Protein Metabolism of the Nervous System*, A. Lajtha, Ed. (Plenum Press, New York, 1969), p. 699.
13. A. A. Smith, M. Karmin, J. Gavitt, *Biochem. Pharmacol.* **15**, 1877 (1966); B. M. Cox, M. Ginsburg, O. H. Osman, *Brit. J. Pharmacol. Chemother.* **33**, 245 (1968).
14. Supported in part by PHS grant NIMH 14013. We thank Dr. L. Coté for assistance in the catecholamine assay.

9 March 1970

Chemical Inducers of Oviposition for the Corn Earworm, Heliothis zea (Boddie)

Abstract. *The corn earworm moth lays its eggs in the vicinity of triacetin, an ingredient of felt-tipped marking pens. Related compounds also induce this behavior. A bioassay was devised to measure the activity of chemicals as oviposition inducers.*

Gravid insects, usually of the order Diptera (flies), have been induced to lay their eggs in the vicinity of certain chemicals. Some compounds eliciting this response are associated with decaying proteinaceous matter such as ammonia, its salts, or amines (*1*), and some sulfur-containing materials such as mercaptans and sulfides (*2*). In one instance aliphatic monoesters (found in food flavors) were reported to be oviposition attractants for *Aedes aegypti* mosquitoes (*3*). In another, chemicals inducing phytophagous insects to oviposit were ones present in the preferred host plants (*4*). Thus, these chemicals may be correlated with a source of sustenance for the young larvae that emerge from the eggs oviposited by the adult (*5*). Chemicals that actively induce oviposition might be useful as a means of controlling insect pests because larvae emerging from eggs laid near an appropriately placed chemical would not find the food normally present and would perish. An attractive feature of this approach is that no toxic chemicals need be used.

In 1968, as part of a program for large-scale rearing of the corn earworm, *Heliothis zea* (Boddie), we began to use felt-tipped marking pens to number cloths on which the insects oviposit. Shortly thereafter we noted that the moths laid more eggs near the ink markings than elsewhere on the cloth. The active ingredient in the ink was isolated and identified as triacetin.

The first felt-tipped pens to induce the oviposition response were manufactured by the Zip Mark Corp., Bordentown, N.J. (*6*). Subsequent tests showed that Aqua Mark (Chemolene Industries, Bordentown, N.J.) and Marks-A-Lot (Carter's Ink Co., Cambridge, Mass.) pens also produced active markings.

The pure active principle in the ink wick was isolated after a series of exploratory experiments in which fractions found active by bioassay were progressively purified. The following procedure worked well. Two ink wicks were sliced into disks about 5 mm thick and allowed to dry overnight (*7*). The wick disks were then extracted in a Soxhlet apparatus with 100 ml of ethyl ether for 3 hours. The ether extract was washed twice with 50-ml portions of distilled water, dried over anhydrous sodium sulfate, decolorized with activated charcoal, and finally concentrated to 1 to 2 ml. Chromatography of this ether extract on a column of silica gel (*8*) with a mixture of ether and hexane (1 : 1) as eluant gave active material between 40 and 140 ml. Gas chromatography of the active material on an OV-17 column (*9*) disclosed the presence of two compounds. Only one was active, and it comprised 27 percent of the material based on peak area. The active fraction from the silica gel column was concentrated to a few milliliters and chromatographed again on silica gel as already described except that the column was developed serially with 100 ml each of 10, 20, 30, 40, and 50 percent ether in hexane by volume. The inactive component was found in the 10 percent eluate and the active one in the 30 percent fraction. Yield of active compound was 20 mg per wick.

The infrared spectrum of the compound showed prominent characteristic acetate absorption (1745 and 1220 cm^{-1}). The nuclear magnetic resonance (NMR) spectrum of the compound in CCl_4 was obtained on an HA-100 Varian spectrometer. It showed an intense singlet at 2.01 parts per million (ppm) and two multiplets centered at 4.13 and 5.11 ppm (from tetramethylsilane) with respective proton absorptions of 81 : 36 : 8. The singlet at 2.01 ppm was undoubtedly due to acetate protons, and the multiplets were tentatively assigned to -C*H*-O- protons. These data suggested the compound was a triacetate; that is, if the multiplet at 5.11 ppm were one proton, the acetate absorption would be equivalent to roughly nine protons, or three acetate groups. Triacetin, with a proton ratio of 9 : 4 : 1 (acetate, primary and secondary protons), fit the 81 : 36 : 8 proton ratio very well. Comparison of the infrared and NMR spectrums of authentic triacetin with the corresponding spectrums of the active compound from the wick showed that they were virtually the same and that the active substance was indeed tri-

Table 1. Oviposition ratio (eggs in treated area divided by eggs in untreated area) for chemicals related to triacetin and for triacetin determined concurrently at two concentrations.

Chemical	Oviposition ratio			
	Chemical at		Triacetin at	
	0.1 mg/ml	1.0 mg/ml	0.1 mg/ml	1.0 mg/ml
Diacetin	1.00	3.84	3.82	3.24
1-Monoacetin	0.99	1.84	2.29	5.13
Tripropionin	2.40	3.44	3.82	3.24
Dipropionin	2.64	4.70	3.38	5.92
Monopropionin	1.26	1.42	3.38	5.92
Tributyrin	1.28	3.88	3.82	3.24
Ethylene glycol, diacetate	1.01	1.50	2.29	5.13
1,3-Propanediol, diacetate	0.85	1.34	3.82	3.24
1,3-Butanediol, diformate	1.18	1.81	3.38	5.92