rial which would modify the shape of the erythrocyte and the elastic characteristics of the membrane, resulting in its destruction (*15*).

The observation that there is a slightly lower frequency of gene P^a (although statistically not significant) among the lowland Sardinian population than among the highland population (*9*) is confirmed by our data on individuals of the Oristano and Tortolì areas (our Sardinian control groups) and is in line with association between favism and acid phosphatase phenotypes. In fact, in Sardinian lowlanders the frequency of the Gd^{Med} gene is higher than it is in the highlanders; therefore the P^a gene could have undergone a selective negative pressure in the lowland areas.

As far as we know, ours is the first report showing that alleles of a gene coding for an enzyme polymorphic in all human populations affect the fitness of the involved phenotypes in special genotypic [Gd(−),Med phenotype] and nongenotypic conditions (ingestion of fava beans). Other similar examples refer to some genetic polymorphisms with limited diffusion [Gd(−), HbS, and so on] and to the association between blood groups and infectious diseases (*16*). On the contrary, the association of blood groups with internal diseases, because the selective effect involves mainly individuals past the reproductive age, is not likely to influence gene frequencies.

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4. Red cell acid phosphatase is an enzyme dependent on sulfhydryl groups, with an electrophoretic polymorphism determined by the occurrence of three common alleles: P^a, P^b, and P^c at an autosomal locus; correspondingly there are six electrophoretic phenotypes: A, B, C, BA, CA, and CB (*5*).
5. D. A. Hopkinson, N. Spencer, H. Harris, *Nature* **199**, 969 (1963).
6. A search for such association was strongly prompted by previous in vitro studies (*7, 8*) in which we demonstrated that red blood cell acid phosphatase is highly susceptible to the inactivating action of oxidized glutathione and acetylphenylhydrazine and that the isoenzymes of acid phosphatase show a different resistance toward these substances. As a conclusion to the last study in the series, we stated, "From a more general standpoint one wonders whether the differential liability of isoenzyme fractions towards toxic agents could result in a differential fitness in favour of the genotypes bearing the most stable combination of isoenzymes." Under these circumstances the a priori probability of an association between hemolytic event and phosphatasic phenotype appeared to be definitely not negligible, in contrast with the situation of most of the similar associations reported in the literature (*8a*).
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10. According to the "Nomenclature of Glucose-6-Phosphate Dehydrogenase in Man" (*ibid.*, p. 545), the male phenotype with the normal enzyme is designated by Gd(+),B. The male phenotype with a deficient enzyme having an electrophoretic mobility similar to B is designated by Gd(−),Mediterranean, and the corresponding allele is designated by $Gd^{Mediterranean}$.
11. Prof. Harry Harris has told us that Dr. Hopkinson, who has studied 29 Greek subjects with favism, obtained data showing an excess of P^a gene. Although not significant, this result, obtained independently from ours in a third population, can be regarded as a confirmation of the association between hemolytic favism and acid phosphatase phenotype.
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17. The two girls admitted in our clinic for favism are not included: both showed B phenotype for acid phosphatase. Fourteen females from Sardinia showed an intermediate G-6-PD deficiency.
18. Subjects deficient in G-6-PD were not tested for thalassemia. Values for normal subjects are those reported by Modiano *et al.* (*9*).
19. We thank Prof. Harry Harris for helpful discussion. This work was supported by the Italian National Research Council.

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Electrical Activity of the Hypothalamus: Effects of Intraventricular Catecholamines

Abstract. *The injection of epinephrine into the third ventricle of the rat brain causes a biphasic elevation and depression in the integrated multiple-unit electrical activity of the median eminence. Activity in the arcuate nucleus decreases after the injection of the catecholamines. These changes in the integrated multiple-unit electrical activity may be related to the secretion of hormones by the anterior pituitary gland.*

The hypothesis that brain catecholamines play an important role in the regulation of the secretion of hormones from the anterior pituitary is strongly supported (*1*). Catecholamines have been administered intraventricularly to bypass the limited permeability of the blood-brain barrier to the amines. The injection of epinephrine into the third ventricle of the estrous rabbit or the pentobarbital-blocked proestrous rat induces ovulation (*2*). Intraventricular injection of dopamine causes the release of follicle-stimulating hormone and luteinizing hormone in the male rat (*3*). Norepinephrine inhibits stress-induced release of adrenocorticotropin when injected into the third ventricle of the dog (*4*). Norepinephrine increases the secretion of growth-hor-

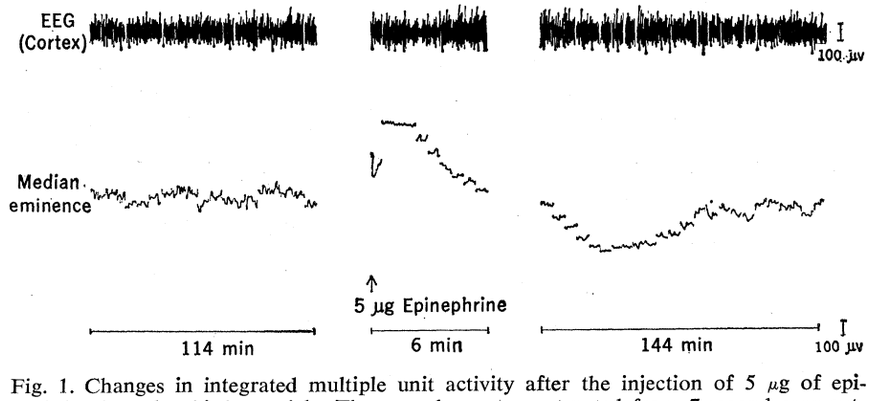

Fig. 1. Changes in integrated multiple unit activity after the injection of 5 µg of epinephrine into the third ventricle. The record was reconstructed from 7-second segments extracted from a continuous record at successive 6-minute intervals. For the first 6 minutes after the injection of the catecholamine, however, 7-second segments were extracted every 30 seconds.

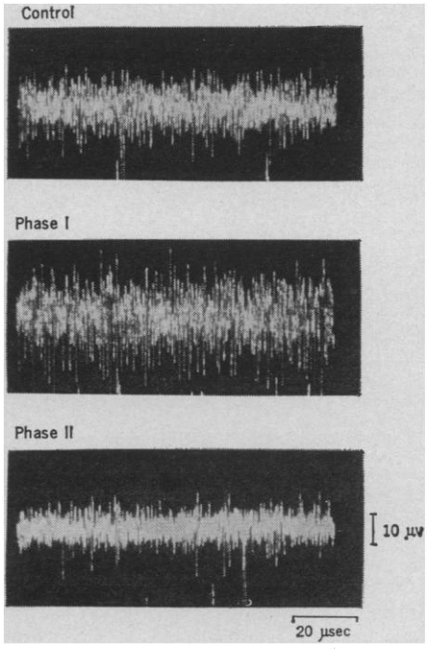

Fig. 2. Photographs of oscilloscopic records during the control period; phase I, approximately 30 seconds after the injection of the epinephrine; and phase II, 30 minutes after the injection.

mone releasing factor when injected into the lateral ventricle of the rat (*5*). We now report that the injection of catecholamines into the third ventricle of the rat brain causes repeatable changes in electrical activity of the median eminence and arcuate nucleus areas known to be involved in the regulation of the secretion of anterior pituitary hormones.

Sprague-Dawley rats ovariectomized 1 to 3 weeks earlier were injected with 5 μg of estradiol benzoate per day for 2 days prior to recording. Animals were anesthetized with urethane and fixed in a stereotaxic frame. A 22-gauge stainless steel outer cannula was stereotaxically inserted into the third ventricle and fixed with dental cement. The cannula was inserted at a 19-deg angle from the perpendicular in the median plane to allow placement of large microelectrodes (tip diameter, 20 to 50 μm) in the basal hypothalamus. Integrated multiple-unit activity was recorded as previously described (*6*). Cortical electroencephalogram, electrocardiogram, respiration, and, in a limited number of cases, blood pressure were monitored. Catecholamines were injected into the third ventricle through a 28-gauge inner cannula in a volume of 3 μl of saline solution. Electrode placements were histologically confirmed by the Prussian blue reaction for iron deposited at the electrode tip by direct current.

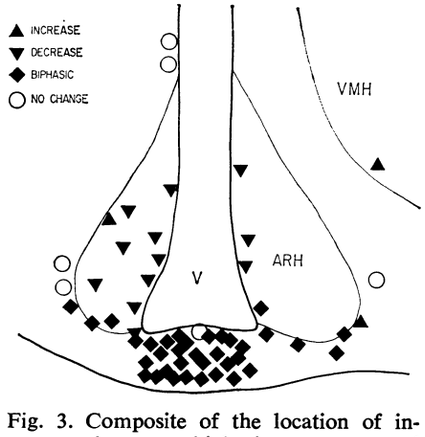

Fig. 3. Composite of the location of increases, decreases, biphasic responses, and no changes in multiple unit activity in the hypothalamus after the intraventricular injection of epinephrine.

The injection of epinephrine in amounts from 1 to 5 μg caused a highly repeatable biphasic change in neural activity in the median eminence (Fig. 1). A typical response consisted of an elevated multiple-unit activity lasting from 3 to 6 minutes followed by a period of depressed activity lasting from 30 to 120 minutes. Photographs of actual oscilloscopic records during (i) the control period, (ii) approximately 30 seconds after the injection of the epinephrine, and (iii) 30 minutes after the catecholamine are shown in Fig. 2. In 27 out of 28 placements of electrodes in the median eminence a biphasic response was observed (Fig. 3). A similar response was seen in nine cases in which the electrode was located between the border of the arcuate nucleus and the median eminence. Of the catecholamines tested epinephrine was the most potent in evoking a biphasic response. Norepinephrine was slightly less potent whereas dopamine was effective only at higher doses. Isoproteronol, however, caused only an immediate decrease in multiple-unit activity in the median eminence. In 12 out of 13 cases in which the electrode was located in the arcuate nucleus above the lateral recess of the third ventricle the injection of epinephrine caused only a decrease in multiple-unit activity (Fig. 3). No consistent change in neural activity was observed in the ventral hippocampus or in other areas of the hypothalamus not shown in Fig. 3. Neither the injection of acid saline solution nor of tartaric acid into the third ventricle affected multiple-unit activity in any of the areas recorded from. The changes in neural activity in the arcuate nucleus and median eminence were independent of changes in electroencephalogram, blood pressure, and respiration.

The localization of these consistent changes in the neural activity of the arcuate nucleus and median eminence suggests that they may be related to the control of secretion of hormones from the anterior pituitary. These areas are known to contain the cell bodies or axons of neurons containing hypophyseal releasing factors (*7*). Changes in multiple-unit activity in the arcuate nucleus and median eminence have been shown to be correlated with the stimulation of an ovulatory surge of luteinizing hormone (*8*). However, the possibility cannot be excluded that the observed changes in electrical activity in the median eminence are related to the stimulation of the magnocellular nuclei since the fibers of these nuclei pass to the neurohypophysis through the internal layer of the median eminence (*9*).

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