Further experiments were done with the Colmar ci^D/Cat stock (no change in mean) and two stocks obtained from it by selecting for 6 generations for respectively high and low expression ratio at 25°. Three cultures each of the base population and the 'low' line and two cultures of the 'high' line were reared at 15°, 25° and 30° (Fig. 1). The mean expression of the low line has a direct relation with the change of temperature and the mean expression of the high line an inverse relation. The frequency distributions (Fig. 2) reveal that each selection line reacts uniformly in one direction. The mean of the base population does not change significantly with the change in temperature. However, the frequency distributions suggest an increase in the variation at lower temperatures on either side of the mean, resulting in significantly higher variances at lower temperatures (Tab. II). This can only mean that the Colmar ci^D/Cat base population is heterogeneous and consists of individuals reacting to a change of temperature in different directions. Selection for high or low expression ratio at 25° separates the genotypes with different reaction norms.

Our results demonstrate that the conclusion of HOUSE[7], that 'ci^D ... shows a linear increase in L-4 interruption with increasing temperature' has no general validity. The direction of temperature response is not determined by the properties of the ci^D gene. The reaction is governed by the whole developmental system of wing venation including the influence of the ci^D gene. Therefore it is completely dependent on the genetic background.

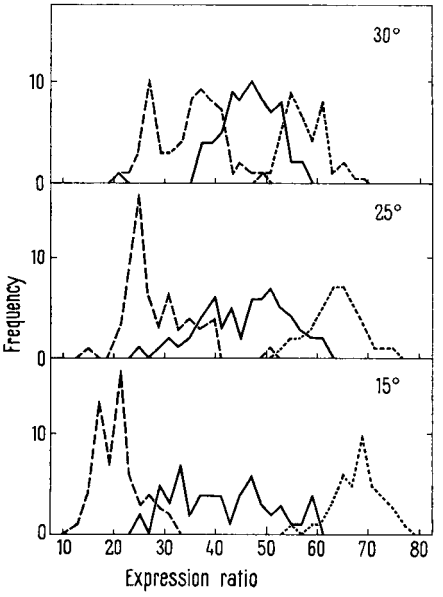

Fig. 2. Frequency distributions at 3 different temperatures of the Colmar ci^D/Cat base population (solid lines) and the lines selected for low (broken line) and high (stippled lines) expression ratios. Only the distributions of ♀♀ are given.

Tab. II. Variances of the Colmar ci^D/Cat base population at 3 different temperatures. The variances are pooled from 3 cultures, and P was calculated following Bartlet's test.

	Variances			P for homogeneity
	30°	25°	15°	
♀	30.1	59.3	113.6	< 0.001
♂	27.3	60.5	87.0	< 0.001

The question arises whether this conclusion has general validity. Only after performing the experiments, did I find that KAMSHILOW[10], as early as 1939, had published some experiments done with the same theoretical background, which seem to have been overlooked. Using the phenotypic expression of the *Drosophila* mutant eyeless as influenced by humidity of the culture media, he succeeded in selecting for a change in direction of the phenotypic reaction. Moreover KAMSHILOW[10] in eyeless, and recently WADDINGTON[11] in several other mutants, found that selection can change the quantitative relationship between temperature and expression.

The results reported here indicate that temperature experiments on morphological mutant traits have no more value in elucidating gene action than experiments on any normal morphological character. Environment acts on a development system arising as an integrated result of a complex interplay of many genetic factors. This kind of experiment can only tell something about the properties of such systems as a whole. In addition, these experiments provide a clear demonstration of interaction between genotype and environment in the sense in which this term is used in quantitative genetics (ROBERTSON[12]).

Zusammenfassung. Durch Versuche mit der Mutante ci^D von Drosophila wurde nachgewiesen, dass die temperaturabhängigen Veränderungen in der Ausprägungsweise ausschliesslich durch den Restgenotypus bestimmt werden. Diese Feststellung hat Bedeutung für Genphysiologie, Evolution und quantitative Vererbung.

W. SCHARLOO[13]

Genetisch Laboratorium Leiden (The Netherlands), December 22, 1960.

[10] M. M. KAMSHILOW, C. R. Acad. Sci. URSS *23*, 302 (1939).
[11] C. H. WADDINGTON, Genetical Research *1*, 140 (1960).
[12] F. W. ROBERTSON, Genetical Research *1*, 288 (1960).
[13] I should like to thank Prof. H. GLOOR for his critical interest and Prof. H. R. VAN DER VAART of the Instituut voor Theoretische Biologie, Leiden, for advice in statistical matters.

Action of Reserpine on the Submicroscopic Morphology of the Pineal Gland[1]

In a previous paper[2], it was demonstrated that the main morphological characteristic of the *pinealocyte*, the parenchymal cell of the pineal gland, is represented by club-shaped perivascular expansions connected to the cell edge by thin pedicles. These expansions contain mitochondria and two types of vesicles: one having an homogeneous light content and the other an heterogeneous one, with a central osmium deposit (Fig. 1). The mean diameter of the two types of vesicles is of 410 Å while the dense granules have a mean diameter of 210 Å. The existence of a single distribution curve for the two types of vesicles was considered as an indication that there may be intermediary forms between them (Fig. 3). The name *plurivesicular secretory processes* was proposed for these cellular expansions emphasizing their probable function in the pineal gland.

[1] This research has been supported by the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina and Grant B 1549 of the National Institutes of Health, United States Health Service.
[2] E. DE ROBERTIS and A. PELLEGRINO DE IRALDI, sent for publication.

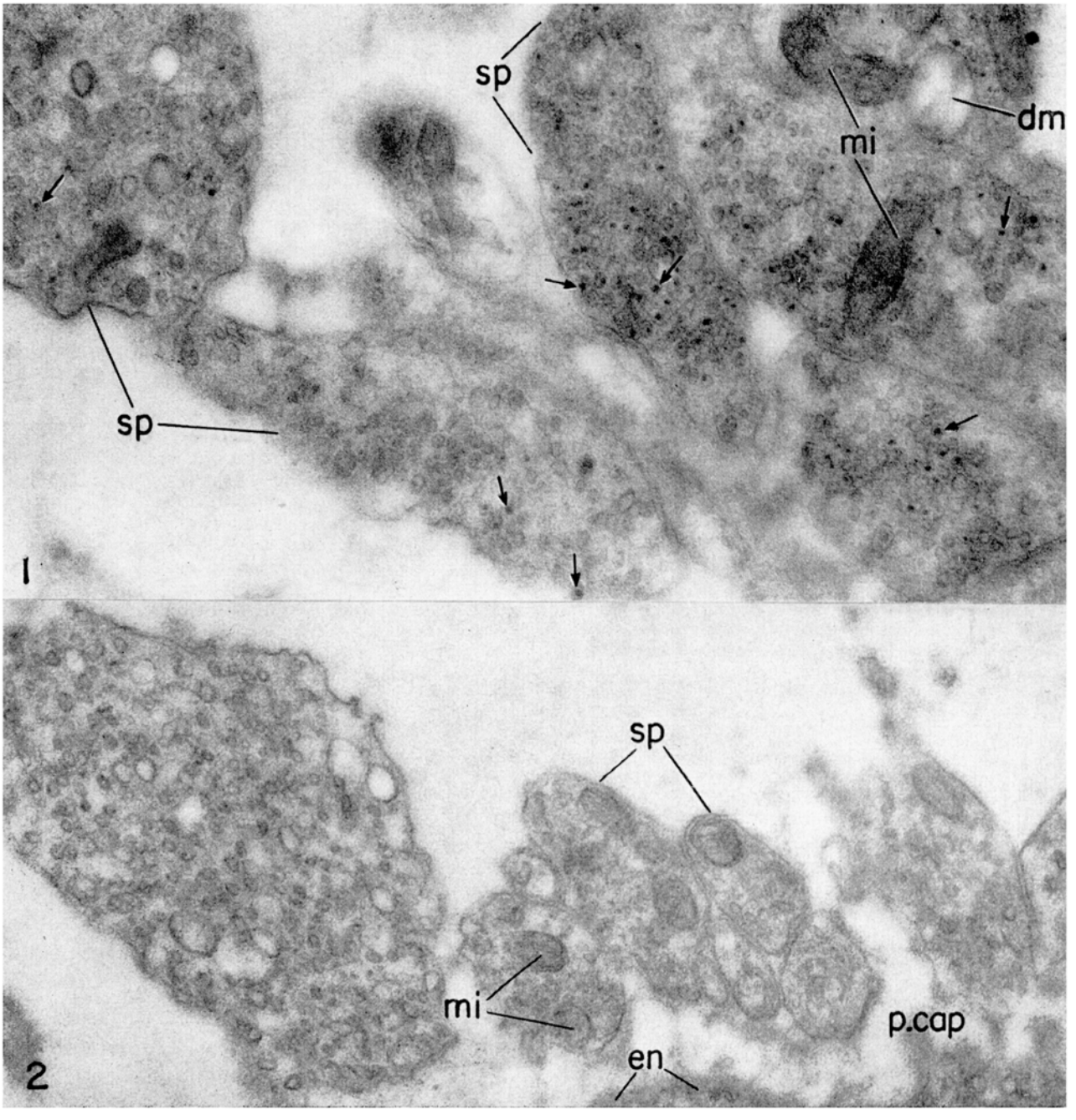

Fig. 1. Electronmicrograph of several plurivesicular secretory processes of the pineal gland (*sp*) of a normal control rat. Numerous vesicles with light homogeneous content and others with a dark osmium granule (arrows) make the main content of the process. There are also mitochondria (*mi*), some of them showing degeneration (*dm*). ×48000.

Fig. 2. Same as Figure 1; 2 h after injection of reserpine. See the disappearance of all the heterogeneous vesicles and the reduction of size of the vesicular material. *en* = endothelium, *p.cap.* = pericapillary space, *sp* = secretory process. ×48000.

The findings of GIARMAN and DAY[3] of an exceptionally high concentration of catecholamines and serotonin, and of LERNER et al.[4] of the N-acetyl-5-methoxytryptamine —the pineal hormone called *melatonin*—and the fact that some of the biogenic amines reduce osmium tetroxide intensely[5] and can be localized with the electron microscope[6], led us to interprete tentatively the plurivesicular material as the site of storage of some of these amines. To check this hypothesis, reserpine, a substance known to produce the release of serotonin[7], norepinephrin[8] and dopamine[9] from the nerve tissue, was used.

[3] N. J. GIARMAN and M. DAY, Biochem. Pharmacol. *1*, 235 (1958).
[4] A. B. LERNER, J. D. CASE, Y. TAKAHASHI, T. H. LEE, and W. MORI, J. Amer. Chem. Soc. *80*, 2587 (1958).
[5] H. S. BENNETT, Amer. J. Anat. *61*, 333 (1941).
[6] E. DE ROBERTIS and A. VAZ FERREIRA, Exp. Cell Res. *12*, *568* (1957).
[7] P. A. SHORE, A. PLETSCHER, E. G. TOMICH, R. CARLSSON, R. KUNTZMAN, and B. B. BRODIE, Ann. N.Y. Acad. Sci. *66*, 609 (1957).
[8] Å. CARLSSON, E. ROSENGREN, A. BERTLER, and J. NILSSON, in *Psychotropic Drugs* (Ed. S. Garattini and V. Ghetti, Milano 1957), p. 363.
[9] A. BERTLER and E. ROSENGREN, Exper. *15*, 10 (1959).

White rats of 1.5–3 months were injected intraperitoneally with 5 mg/kg of reserpine and sacrificed 10 min to 8 days thereafter. Another group was injected with a similar dosis during four consecutive days and the rats were sacrificed 1 h after the last injection. The preparative techniques for the electron microscope were those previously described[2].

On the electron micrographs, the morphological aspect of the plurivesicular component was studied in the treated animal and compared with that of parallel normal controls. Measurements of the diameter of the vesicles and granules and determinations of size distribution were carried out in the controls and 2 h after reserpine injection (Fig. 3). The relative proportion of homogeneous to heterogeneous vesicles was determined in a total of 500 elements for each particular experiment (Fig. 4).

It was found that reserpine produces the almost complete disappearance of heterogeneous vesicles containing dense granules between 2 and 48 h after a single injection

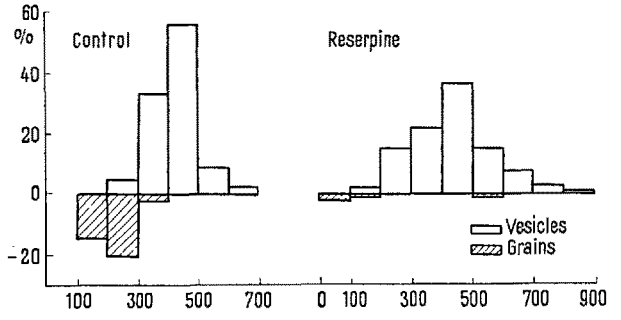

Fig. 3. Histograms of sizes of vesicles in the secretory processes of the control rat and 2 h after injection of reserpine. In the upper part distribution of sizes of 100 vesicles, below distribution of sizes of dense grains/100 vesicles. See the almost disappearance of grains with reserpine (only 22 grains in 600 vesicles).

(compare Fig. 1 and 2). There is a decrease in size of all vesicles (Fig. 3) and some of them resemble sections of the tubular elements found in the pedicle of the expansion[2]. The changes in the relative proportion of the two types of vesicles demonstrate that the drug has an action in as early as 10 min, reaching the maximal effect 2 h after the injection (Fig. 2).

The storage of dense granules was very small after 24 and 48 h increasing notably after 3 days and reaching an almost normal content after the 6th and 8th day. The curve of grain restoration is strikingly similar to that obtained by SHORE and BRODIE[10] with the recovery of serotonin and noradrenaline in the brain of rabbits after injection with reserpine (Fig. 4).

Chronic administration of reserpine leads to the almost complete disappearance of the dense granules and to other profound changes in the vesicular components of the pinealocytic expansion.

All these findings suggest that the secretory processes of the pinealocyte contain the biogenic amines and that these are localized in the plurivesicular material. The fact that reserpine produces the release of serotonin, noradrenaline and dopamine and that these amines may reduce osmium tetroxide, makes a finer morphological discrimination difficult at the present time.

Further pharmacological studies with other drugs that may influence the metabolism of biogenic amines should be carried out. The observations made so far demonstrate that the electron microscope may be a useful tool for studying the sites of storage and mechanisms of release of biogenic amines in the pineal gland.

Zusammenfassung. Pinealdrüsen von Ratten nach Reserpin-Injektion wurden elektronenmikroskopisch untersucht. Die Behandlung ergab auffallende Veränderungen in den plurivaskulären Sekretionsprozessen[2]. Die dichten Bläschen verschwanden innerhalb 2–48 h nach der Reserpingabe nahezu vollständig und erschienen erst wieder zwischen dem 3. bis 8. Tag. Dieser Wechsel verläuft parallel zum Wiederauftreten von Serotonin und Noradrenalin nach SHORE und BRODIE[10].

AMANDA PELLEGRINO DE IRALDI
and E. DE ROBERTIS

Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Buenos Aires (Argentine), November 15, 1960.

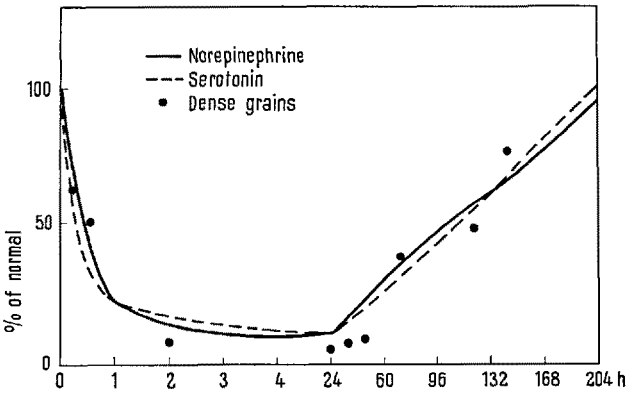

Fig. 4. Curve of SHORE and BRODIE[10] showing the concentration of serotonin and noradrenaline (in % of the normal) in the nervous tissue after a single injection of reserpine. On this curve the points indicate the % change of the normal in dense granules found in our experiments. (100% represent the number of dense grains/100 vesicles found in the normal controls).

[10] P. A. SHORE and B. B. BRODIE, in *Psychotropic Drugs* (Ed. S. Garattini and V. Ghetti, Milano 1957), p. 423.

Tryptophan, Precursor of Tropic Acid in *Datura Stramonium*

Hyoscyamine is the ester of tropine and *l*-tropic acid. Considerable work has been done on the biogenesis of this alkaloid[1]. The pyrrolidine part of tropine has been shown to derive from ornithine[2], but the origin of the residual portion of it has not yet been established[3]. Likewise, the biogenesis of the tropic acid moiety of hyoscyamine has remained in darkness.

Tropic acid – α-phenyl-β-hydroxypropionic acid – has been variously suggested to be of terpenoid origin[4] or to be formed from prephenic acid[5]. LEETE has shown re-

[1] W. O. JAMES, *The Alkaloids*, Vol. 1 (R. H. F. MANSKE and H. L. HOLMES, Academic Press Inc., N.Y. 1950), p. 64.
[2] E. LEETE, L. MARION, and J. D. SPENSER, Can. J. Chem. *32*, 1116 (1954).
[3] A. V. ROBERTSON and L. MARION, Can. J. Chem. *38*, 294 (1960).
[4] E. M. TRAUTNER, Austr. Chem. Inst. J. and Proc. *14*, 411 (1947).
[5] E. WENKERT, Exper. *15*, 165 (1959).