Antigenic structure of staphylococci (*)

ARTUR TORRES PEREIRA

Instituto Bacteriológico Câmara Pestana, University of Lisbon, Portugal

A. – INTRODUCTION. POSING OF THE PROBLEM

The first work published on this matter bore the signature of KOLLE and OTTO [1] and was carried out at the Institute for Infectious Diseases in Berlin, then under the directorship of ROBERT KOCH. It may be stated that, after the lapse of 61 years, the investigations, few and far between, have not been sufficiently enhanced as to their value by text-book authors. One of our difficulties is to explain why the research work had no continuity and its value was not duly appreciated. The few authors interested in the antigenic and serological study of *Staphylococcus* have used the precipitation and agglutination reactions.

JULIANNELLE and WIEGHARD [2,3], THOMPSON and KHORAZO [4], COWAN [5], HOBBS [6], STERN and ELEK [7], and VISCHER [8] were the authors who devoted themselves to antigenic study through precipitation reactions. Most of their work must have been performed with irregularly absorbed sera and nowadays the method has been almost entirely abandoned as regards *Staphylococcus* classification.

The agglutination method, which, as we stated, appeared in 1902, was especially studied by JULIANNELLE [9], HINE [10], COWAN [11], CHRISTIE and KEOGH [12], HOBBS [13], PILLET [14-20], OEDING [21-26] and GRÜN [29-30]. Each of these authors, on completing his investigations, thought he had demonstrated through a varying number of *Staphylococcus aureus* strains isolated from patients, that his method was efficacious in practice to distinguish between these strains and classify them. However, year after year, students of bacteriology have not found this matter duly enhanced in textbooks, and this is still the situation today. One might be led to suppose that a manifest discredit has attended the serological study of staphylococci throughout the years.

The first important modern work is due to COWAN [11], who discarded tube agglutination and adopted slide agglutination instead, which is easier

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to perform and more accurate. Though the reading of the agglutination was effected over a comparatively long period (3 minutes), COWAN came to the conclusion that there are three types of *Staphylococcus aureus*, viz., I, II and III. CHRISTIE and KEOGH ([12]) described six types more which are indicated by means of Arab figures, from 4 to 9, and HOBBS ([13]) increased to 13 the number of standard types.

HOBBS, who rightly held the opinion that in Cowan's technique there were numerous deficiencies preventing the reproduction of the results, failed, however, to bring about the necessary changes in the technique. She tried to prove that each one of the thirteen standard staphylococci may originate a specific serum of a comparatively high titre. This technique was not widely accepted by bacteriologists, and HOBBS herself ended by discarding it.

The conclusion is that COWAN, CHRISTIE and KEOGH, and HOBBS produced useful work using deficient techniques; their merit is great indeed, but at the same time the inaccuracy of the method they adopted prevented its general acceptance.

Between 1950 and 1955 PILLET and collaborators ([14-20]) published several papers without bringing about improvements in serological technique. Their merit consisted in their having considered the existence of a common antigen whose quantity is unequally represented on the surface of several strains of *Staphylococcus*.

Since 1952 PER OEDING has published several papers and has often changed his technique and his concept of classification. Initially (1952) he considered the antigenic group factors *a*, *b*, *c* and *e*, and the specific factors *f*, *h*, *i* and *k*. The main types were represented as follows: *abe*, *ab*, *abc*. In the following year, after the description of O-inagglutinability, OEDING stated that most *Staphylococcus* strains have antigens *h* and *i*. In 1957 he ceased to perform agglutinations with living and with autoclaved bacteria. He started to use living bacteria only, after 5 hours' incubation, because he thought it could be concluded that superficial antigens *i* and *h* develop much more slowly than the deep ones. In 1958 OEDING defined new groups in relation to antigens *e*, *i* and *k*, which he considered as determinants. We believe that this last paper clearly shows OEDING's preoccupation in obtaining many different agglutinations like those exemplified in formulas (abe)k, (aeh)k, aek, a(b)eh(k), aehk, etc. As this technique does not allow of such distinctions, it appears that OEDING's attitude is open to criticism, although he has always been very faithful to the serological method.

B. – PERSONAL SEROLOGICAL TECHNIQUE AND ANTIGENIC ANALYSIS

I. *Antigenic loss variation.* — When about eight years ago we began to study the antigenic analysis of staphylococci, we tried to obtain sera after inoculation in rabbits of various standard strains. We used, besides the thir-

teen international types, numerous recently isolated British and Portuguese strains. Using only immune sera prepared from the thirteen classical types (COWAN, CHRISTIE and KEOGH, HOBBS), and after a variable degree of absorption, we did not succeed in identifying or obtaining agglutinations with recently isolated strains from various pathological processes. Only when we began to use as immune sera-producing types the recently isolated British and Portuguese strains could we easily verify the agglutination of most of the isolated strains in the routine bacteriological examinations.

The phenomenon of *antigen loss variation* which was described in 1961 by TORRES PEREIRA ([31]) explains all the difficulties and misfortunes which made us stumble at the beginning of our work. The phenomenon involves two of the main antigenic factors we described and which we designate by numbers 13 and 17. In brief, the phenomenon consists in the following: most of the recently isolated strains possess either antigen 13 or antigen 17. These strains, after being sub-cultivated many times on nutrient agar slants, lose the described antigen 13 or 17 and usually reveal another one, respectively antigen 3 or antigen 1. Antigen 3 and antigen 1 are the main antigens presented nowadays by the Collection type strains Cowan III and Cowan I. We believe that lack of knowledge of the two variations (17 → 1 and 13 → 3) must have adversely affected the serological work of other authors. Being known that factor 17 exists in over 40 per cent of recently isolated strains and factor 13 in about 20 per cent, it will be readily understood how important the knowledge of antigenic loss variation is for the establishing of any serological technique as regards staphylococci.

TABLE 1.

Loss variation of antigen 13 in recently isolated strains

Strain	Number of subcultures	Original antigenic behaviour	Variation observed after N.° of transfer	Final antigenic behaviour
37593	30	13+ 3—	13	13— 3—
38328	30	13+ 3—	10	13— 3+
41147	30	13+ 3—	11	13— 3+
41355	30	13+ 3—	13	13— 3+
42062	30	13+ 3—	12	13— 3—
42094	50	13+ 3—	7	13— 3+
42136	30	13+ 3—	9	13— 3+
43236	31	13+ 3—	10	13— 3+
45142	40	13+ 3—	9	13— 3+
46112	40	13+ 3—	25	13- 3+
46449	40	13+ 3—	6	13— 3+

TABLE 2.

Phage-typing of pairs of strains of « staphylococcus » that had undergone the loss variation

Strains		Number of subcultures before the final strain	Antigenic constitution (Torres Pereira)				Phagic pattern (R. E. O. Williams)	
			Variation 13 → 3		Variation 17 → 1		Phage's dilution	
Identification	Condition		antigen 13	antigen 3	antigen 17	antigen 1	RTD	RTD × 1.000
38328	Original Final	30	+ —	— +			55/77w Idem	7/42E/47/53/54/73/75/77 Idem
41147	Original Final	30	+ —	— +			83w Idem	83 Idem
41355	Original Final	30	+ —	— +			NT Idem	7/42E/75/77 + (42B/47C) 42B/81 + 47C
42136	Original Final	30	+ —	— +			83w Idem	83 Idem
43346	Original Final	50			+ —	— +	29w 29/52w	29/52/52A/80 Idem
44159	Original Final	52			+ —	— +	NT Idem	73 + w Idem
44320	Original Final	48			+ —	— +	NT Idem	52 NT

In Table 1 this phenomenon, encountered in a group of 11 strains of staphylococci just isolated from pus, sputum, stools and nasal secretions, is described. Generally speaking, about 10 daily transfers were enough to bring about the disappearance of antigen 13. Antigen 3, which appears almost invariably after the disappearance of antigen 13, may represent an alternative in relation to the genotype which is also responsible for antigen 13. We were never able to demonstrate the presence of antigen 3 in an occult form in bacteria with agglutination 13, even though appropriate techniques were used. The same applies to antigens 17 and 1. In nature, strains of *Staphylococcus* would generally be provided with antigen 13 or 17. After antigenic variation, they occur respectively as 3 or 1. This explains why 11 of the strains that constitute our basic set (see further on) made up of 23 *Staphylococcus* strains reveal factor 1, although the latter is only exceptionally found in strains recently isolated from patients. We accept that most of the strains that constitute our basic set were provided, when they were isolated, with factor 17, but in the course of several years and after hundreds of transfers this factor was lost and replaced by factor 1, which apparently is stable.

In collaboration with Prof. R. E. O. WILLIAMS and Dr. PATRICIA JEVONS we further demonstrated through phage typing that the strains which underwent the phenomenon of loss variation did not show any alteration of phage reactions.

Table 2 shows that in 7 pairs of strains that experienced antigenic variation (four 13 → 3 and three 17 → 1) only two presented small differences in the reactions to bacteriophages.

II. *Agglutinin removal sequence.* — Before summing up our serological technique, it is indispensable to draw attention to this other phenomenon, which explains that the agglutinating properties of a serum in relation to

TABLE 3.

Serum anti-Cowan III absorbed with strain Christie and Keogh 8

Absorptions in succession	International types												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Before absorption	+	+	+	+	+	+	+	+	+	+	+	+	+
After 1st absorption . . .	—	+	+	—	+	—	—	—	(+)	+	+	—	?
After 2nd absorption . . .	—	—	+	—	(+)	—	—	—	—	+	—	—	—
After 3rd absorption . . .	—	—	+	—	—	—	—	—	—	+	—	—	—
After 4th absorption . . .	—	—	(+)	—	—	—	—	—	—	(+)	—	—	—

several heterologous strains varied after several successive absorptions using always the same absorbing strains.

In table 3 we find an instance of this phenomenon which is responsible for the fact that different lots of the same immune serum reveal, after absorption, different agglutinating properties. This fact may have been at the root of many failures in the work of several serologists. In order to characterize an absorbed serum it is not enough, therefore, to refer to its behaviour in relation to the homologous and to the absorbent strains. For this reason it became necessary to standardize the absorption technique. For this purpose, we have defined a basic set composed of 23 *Staphylococcus* strains, each specific serum being considered as ready for use if it agglutinates only certain bacteria of the basic set.

As may be seen from table 3, after the first absorption it may be considered that the serum has been absorbed in relation to the absorbing strain CHRISTIE and KEOGH 8. However, as we perform new absorptions, always with the same absorbing strain, we note that other heterologous agglutinations disappear gradually.

At the end, we obtained in some cases really specific sera, while in others all the agglutinins of the sera are exhausted. This is yet another example of the difficulties of the serological technique of the staphylococci.

III. *Preparation of immune sera.* — For the preparation of immune sera the first inoculations of the rabbits are done with formalin-killed suspensions of *Staphylococcus* and later with living suspensions. Absorbed sera are always used since, generally speaking, all immune sera, and even the sera obtained from non-inoculated rabbits, agglutinate most *Staphylococcus* strains, although different dilutions may be needed. The absorptions are always done with serum diluted to 1:5 and are effected with living bacteria in a water bath at 50° C for four hours. In order to consider a serum as absorbed, it is indispensable that we define the properties we ascribe to it. To define the technical conditions of absorption, we selected a set of bacteria to which we gave the name of basic set, constituted by 23 strains. We only consider a serum as absorbed and ready for use in agglutination tests when it behaves in a defined way in relation to the bacteria of the basic set independently of what regards the agglutinations with the homologous strain and the absorbent strain. Thus, when, after the first absorption, a serum ceases to agglutinate the absorbent strain, we go on carrying out new absorptions until the behaviour of the serum in relation to the basic set is the one we defined. At the start, the basic set was constituted only by the thirteen international standard strains, but later on it was constituted by 23 strains to which we gave the following numbers ([32]): 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 22, 29, 33, 38 and 50. Thus, for instance, a specific serum for factor 3

is considered as prepared when, after one or many absorptions, it only agglutinates strains 3, 33, 38 and 50.

IV. *Agglutination technique.* — After some experience with the technique of tube agglutination, we have discarded it and adopted exclusively the slide technique, which provides reproducible results when investigating strong and immediate agglutination reactions which must not exceed 30 seconds.

V. *Identifiable antigenic factors.* — The following are the antigens we have identified with the present technique, in which the personal factor is practically negligible: 1, 2, 3, 7, 11, 13, 17, 18 and 51. These figures regard the standard strains. The first six are identified with sera obtained with classical standard strains: COWAN I, COWAN II, COWAN III, CHRISTIE and KEOGH 7, HOBBS 11 and HOBBS 13, and the three last regard strains isolated in Portugal, the two first from staphylococcal pneumopathies and the latter from urinary infection.

We represent the absorptions by an expression of the *a*/*b* type where *a* stands for the serum to be absorbed and *b* for the absorbent strain. The figures that follow the said expression indicate the standard strains with which the serum still agglutinates at the moment when it is considered as adequately absorbed.

The following are the normal results of the current absorptions:

I/33 = 1, 4, 5, 6, 8, (10), (11), (12), 14, 15, 16, (17), 22.
II/33 = 2, (9).
III/7 = 3, (33), 38, 50.
7/22 = 7, 9.
11/13 = (10), 11, 12.
13/III = (6), (7), (8), (13), (29).
17/I = (5), (14), (17).
18/33 = 18.
51/33 = 51.

The value of all these absorbed sera to identify the antigens of the just isolated strains is very unequal. With the exception of antigen 51, which could only be observed in the strains responsible for urinary infections (TORRES PEREIRA [33]), only antigenic factors 13, 17 and 18 are important among all the other antigenic factors. Twenty per cent of recently isolated strains show antigen 13, 40 per cent antigen 17, about 10 per cent (in intra-hospital conditions) antigen 18; 20 per cent cannot be classified, and the remaining 10 per cent present other antigens.

Antigen 51 is encountered as a rule in the strains of *Staphylococcus albus* that give rise to pyuria.

VI. *Representative serological types.* — Table 4 indicates the representative serological types.

TABLE 4.

Serological « Types »

Strains	Origin	Serological « Type » Per Oeding	Phage « Typing » R.E.O. Williams	Serological « Type » Torres Pereira
37431	urine	acehik	7/77 +	1
37789	pus	abcehik	42B/47C/81	1
35955	blood and blood marrow same	a(b)ceik	81	1
35956	patient	ace(h)ik	42B/47C/81	1
37628	pharynx	a(b)cehik	52/80 +	17
37669	pus	ace(h)ik	47C +	17
34561	pus	a(b)cehik	NT	17
36477	sputum	acehik	29/52/52A/80	17
35146	pharynx		NT	3
35692	urine	abch	83vw	3
34900	wound	abcei	54w	3
37593	stools and urine	abc	42B/47C +	13
37594	same patient	abc(h)	47/53/75/77+w	13
37664	nose	(bi)	29vw	13
28/1	eye	NT	83	18
28/2	pharynx	NT	83	18
28/3	nose	NT	83	18
28/5	blood	NT	83	18
28/16	pharynx	NT	83	18
28/21	eye	NT	83	18

This table deals with the classification of 20 strains. Since these strains were not grouped according to their common origin, it has not been possible to reach an agreement among the results of OEDING, WILLIAMS and our own.

In this table we describe 5 different factors only, whereas OEDING presents 8 or 9 different antigenic formulas, and WILLIAMS 11 bacteriophage types.

The first 883 *Staphylococcus aureus* strains that we studied had the following distribution for the various antigens we succeeded in identifying: 34.3 per cent (antigen 17); 23.7 per cent (antigen 13); 13 per cent (antigen 18); 6.7 per cent (antigen 1); 3.9 per cent (antigen 3); other reactions or non-classified strains 17.9 per cent.

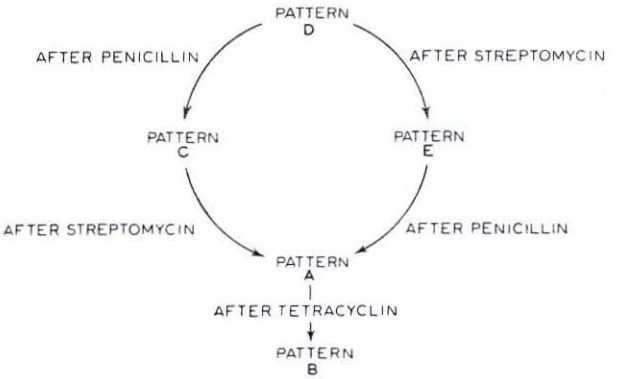
C. - CORRESPONDENCE BETWEEN ANTIGENIC CONSTITUTION AND SENSITIVITY TO ANTIBIOTICS

The number of sensitivity patterns can be restricted to 6, in which are included all the 2,143 *Staphylococcus* strains studied from 1955 to 1960. *Pattern A* bacteria reveal resistance only to penicillin and streptomycin. If a strain reveals sensitivity to chloramphenicol, erythromycin and novobiocin it is included in *pattern B*. In *pattern C*, the bacteria are resistant to penicillin only, in *pattern D* they are sensitive to all antibiotics, and in *pattern E* they are resistant to streptomycin only. *Pattern F*, which is rarely found, reveals resistance to the three tetracyclins only. All those patterns can show exceptions ([32]).

The main patterns defined by us may be dynamically related as observed in Table 5.

TABLE 5.

Origin of different sensitivity patterns from heterogeneous «Staphylococcus» populations



Thus, a strain originally sensitive to all antibiotics (pattern D) may eventually originate all the other patterns. After contact with penicillin, pattern C may appear, and from this one, after contact with streptomycin, pattern A. If the strain is initially in contact with streptomycin, pattern E occurs, and from the latter, in its turn, pattern A is derived after contact with penicillin. From pattern A, which thus had its origin in pattern D (after passing through pattern C or pattern E), pattern B may finally result after contact with tetracyclin. In all cases, it is always a question of a selective action and of the attainment of less heterogenous populations starting from others which are more heterogeneous.

Antigen 18 was found in 82 *Staphylococcus aureus* strains, and all without exception belong to combination 18B. Referring to the above-propounded model of versatility of sensitivity to antibiotics, and since we have never come across combinations 18D, 18C, 18E and 18A, we conclude that bacteria having antigen 18 are necessarily resistant to penicillin, streptomycin and tetracycline.

Applying to *antigen 17* the scheme of versatility of antibiotic sensitivity, we obtained the following percentages for the various combinations: 17D, 19.1 per cent of the strains with antigenic factor 17; 17C, 13.1 per cent; 17E, 6 per cent; 17A, 50 per cent; and 17B, 11.6 per cent. In conclusion, about 20 per cent of the bacteria retain their original type of sensitivity (17D); the combinations that occur thereafter – 17C and 17E – would be comparatively unstable, and bacteria originally resistant to penicillin or to streptomycin would quickly become resistant to both antibiotics. These strains would therefore result in combination 17A, the most frequent one. After this combination, only a comparatively small number of strains – about 10 per cent – would reach the last stage in the versatility process designated as combination 17B. Bacteria with antigen 17 would thus be generally resistant to penicillin and to streptomycin at the same time, but not to the tetracyclines.

With *antigen 13* we found the following percentages for each combination: 13D, 5.1 per cent of strains having antigen 13; 13C, 3.6 per cent; 13E, 5.1 per cent; 13A, 16.1 per cent; 13B, 69.8 per cent. The conclusions in this case are different from those reached for factor 17. We concluded that bacteria having antigen 13 quickly reach the last stage in the versatility process, and only 5.1 per cent of the strains retain their original property (13D). Generally speaking, therefore, bacteria with antigen 13 would reveal resistance to penicillin, streptomycin and tetracycline.

We suggest that the capacity of *Staphylococcus aureus* strains for altering their sensitivity to antibiotics is partly conditioned by their antigenic constitution.

D. – CORRESPONDENCE BETWEEN ANTIGENIC CONSTITUTION AND PHAGE-TYPING

Table 6 will show the correspondence between antigenic constitution and phage-typing.

TABLE 6.

Correspondence between antigenic structure and phage-typing in 209 strains of « Staphylococcus aureus »

ANTIGENIC STRUCTURE	PHAGE GROUPS				Total
	I	II	III	IV or NT	
Antigenic factor 1 or 17 . . .	58	13	21	4	96
Antigenic factor 3 or 13 . . .	7	11	69	5	92
Other antigens or antigens non identifiable	2	1	14	4	21
Total . . .	67	25	104	13	209

In conclusion, it may be stated that 60 per cent (58 in 96) of the strains with *factor 1 or 17* belonged to group I of phage-typing, and 86 per cent of the strains (58 in 67) of phage group I presented one of antigenic factors 1 or 17.

Similarly, 75 per cent of the strains (69 in 92) with one of factors 3 or 13 were classified in phage-typing group III, and 66 per cent of group III strains (69 in 104) revealed one of those two antigens. It appears, therefore, that the correspondence between antigens 17 and 13 and phage groups I and III respectively is marked.

In the research work we are pursuing in Rome at the Microbiology Department of the Istituto Superiore di Sanità and with the cooperation of V. Ortali, we tried to throw some more light on these relationships. It is known that there are different serological groups in the basic set of the phages of the *Staphylococcus* originally described by WILLIAMS and RIPPON ([34]). Thus, phages 29, 52, 52A, 79, 80, 55, 71, 53 and 44A belong

TABLE 7

Correlation between lysogeny and antigenic structure of « Staphylococcus »

Serological group B phages	Staphylococci with antigen 1 or 17 (propagating strains *)	Serological group A phages	Staphylococci with antigen 3 or 13 (propagating strains **)
29	29	3A	—
52	52	3B	—
52A	52A	3C	3C
79	79	6	6
80	80	7	7
—	81	42E	42E
—	3A	47	47
—	3B	—	53
55	55	54	54
71	71	73	73
53	—	75	75
44A	—	—	77
—	—	—	42D
—	—	—	44A

* Carriers of serological group A prophage ?
** Carriers of serological group B prophage ?

to *serological group B*. Phages 3A, 3B, 3C, 6, 7, 42E, 47, 54, 73 and 75 belong to *serological group A*.

The *Staphylococcus* strains corresponding to these phages (propagating strains) and which bear the same number are for the most part classifiable with our agglutinant sera. If we consider as a group the ones that present antigen 1 or 17, and as another group the ones with antigens 3 or 13, we may distribute these strains as follows. Strains with antigens 1 or 17: 29, 52, 52A, 79, 80, 81, 3A, 3B, 55, 71; strains with antigens 3 or 13: 3C, 6, 7, 42E, 47, 53, 54, 73, 75, 77, 42D, 44A.

It seems, therefore, that, though there are exceptions, the phages of *serological group B* are related to *antigens 1 and 17*, and the phages of *serological group A* to *antigens 3 and 13*.

If we accept that the lysogenic strains of staphylococci contain prophages of a different serological group from one to which they are sensitive, and since the main prophages must belong to serological groups A and B, we may admit – speculatively as yet – that the *Staphylococcus* strains having antigenic factors 1 or 17 and generally sensitive to group B phages may carry a prophage of serological group A. The staphylococcus strains would thus have a prophage of *serological group A*, and *antigen 1 or 17* on the surface. Similarly, the strains having in the genoma a prophage of serological group B would present one of antigens 3 or 13. These relationships are summed up in Table 7.

In the Salmonella group lysogenic conversion phenomena have been reported by UETAKE and collaborators ([35]). After lysogenization with phage E 15 *Salmonella anatum* loses the somatic antigen 10 and acquires somatic antigen 15 of *Salmonella newington*. So far we have not succeeded in ascertaining if a phenomenon of this type may be involved in cases of staphylococci with prophages of serological group A or B and which yield antigen 1-17 and 3-13.

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Metabolism and function studied in single neurones (*)

Ezio GIACOBINI

Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

Neurophysiologists no longer doubt that the process of excitation in the nerve cell is accompanied by a series of biochemical events and consumption of energy supplying material. However, until a few years ago discussions on nerve function displayed the feeling that « these phenomena did not belong to the material universe ». This opinion prevailed in spite of early and repeated warnings from several biochemists and biophysicists like Lillie, Erlanger, Gasser and Hill. Many years ago Lillie considered it « self evident that the energy of bioelectric current represents the transformed energy of chemical reaction ». Erlanger and Gasser « compared the electrical phenomena to the ticking of a clock, which indicates that it works but fails to explain the mechanism that makes it work », and Hill expressed his conviction that « the machinery itself is chemical in nature, the fuel it uses is chemical, the acid and plates of the accumulator are chemical and the free energy of chemical change provides the mechanical work ». A considerable amount of data has been accumulated from studies on whole brain preparations, homogenates of nerve tissue, cortex slices, isolated ganglions, or single axons describing biochemical changes during excitation induced by physiological, electrical, ionic or pharmacological stimulation. Besides the well known modification of glucose and lactate levels and an increase in inorganic phosphate resulting from the breakdown of organic phosphates (ATP and creatine phosphate), other biochemical events occur during activity which have been described as follows:

1. Oxidation of glucose, aminoacids or lipids, breakdown of organic phosphates (energy metabolism).
2. Accelerated turnover of proteins and nucleoproteins.
3. Denaturation of proteins: unmasking of the side groups of aminoacid residues (*transconformation and reconformation, paranecrosis*).

(*) Conferenza tenuta nell'Istituto Superiore di Sanità il 26 ottobre 1964.

4. Na^+ and K^+ activation of enzymes (*ATPases*).
5. Combination of Na^+ or K^+ with phospholipids or glycolipids (*carrier hypothesis*).
6. Release, action and subsequent breakdown of specific transmittor substance (*ACh*).

In spite of these results the membrane permeability characteristics of the nerve cell appear to be remarkably stable for a long period of time despite low energy turnover. In the generation of the spike, there is no substantial evidence of an involvement of metabolism other than that required to regulate ionic gradients and membrane potential. One may regard these gradients as a store of potential energy built up through metabolism during an earlier period. This energy is then released in small amounts per impulse during a cyclic series of events that require very little energy other than that derived from the ionic gradients and the associated electric field.

Following the ionic hypothesis of Hodgkin and Huxley, the immediate source for the propagation of the impulse is derived from the concentration batteries for Na^+ and K^+ ions and the metabolic energy is only later required in order to restore the ionic composition. However, according to this hypothesis the ionic flux per impulse is so small compared to the ionic composition of the fiber that even in the absence of a metabolic restorative process many thousands of impulses can be propagated along large nerve fibers without impairing of the mechanism. These assumptions are made on the basis of investigations with so called giant axons, in which powerful metabolic inhibitors such as cyanide, azide or dinitrophenol blocked both sodium extrusion and potassium uptake in the resting state, while having only a slight effect on the sodium movements during the passage of the impulse (HODGKIN & KEYNES, 1954). HODGKIN & KEYNES (1954) concluded from such observations that the permeability system which allows ionic transport across an electrochemical gradient is *not* metabolically dependent as is the secretory system which operates during the recovery phase. Even though there is evidence of increased metabolism, oxygen uptake and increased production of heat during activity in the neural tissue, very little is known about the relationship of these factors to the mechanism of selective permeability barrier and active transport processes both from a quantitative as well as from a qualitative point of view.

The direct observation of intracellular biochemical events and their relationship to the specific function of the nerve cell represents therefore one of the most challenging problems for neurophysiologists today.

With the work of Warburg in 1923, which made it possible to measure the respiratory activity and other metabolic aspects of the brain tissue, a

new possibility was opened. Since then at least three major and independent approaches have been directed toward this goal in neurochemistry :

A. Manometric or respirometric determinations in slices of cerebral tissue or excised sympathetic ganglia electrically stimulated (Mc Ilwain *et al.*, 1951-1962 ; Larrabee *et al.*, 1952-1962).

B. Microfluorimetry of pyridine nucleotides of small exposed surfaces of the brain *in situ* or of single neurones (Chance *et al.*, 1962 ; Terzuolo *et al.*, 1963).

C. Micromanometric determination of enzyme activity, substrates and respiration in an isolated nerve cell preparation at rest and in activity (Giacobini *et al.*, 1963).

Regarding the first approach (*A*), it must be borne in mind that such preparations do not show all the known electrophysiological responses to stimulation nor the spontaneous activity associated with brain *in vivo* and give very little information at the cellular level. Furthermore, the ionic environment external to the nerve cells cannot be readily controlled.

The second technique (B) is of great interest and possibility but it is still too early to be fully evaluated. As a comment to the third possibility (C), it should be pointed out that among the several methods for cellular biochemistry now available, the cartesian diver technique of Linderström-Lang (1957) is, as far as we know, the only one which permits quantitative chemical assays in an isolated living nerve cell, provided that we can find a single cell preparation which can be used in the diver without changes of its functional activity.

Preserving the unaltered structure or the function of the nerve cell during the chemical assay constitutes one of the most difficult problems of neurochemical analysis. Several basic requirements have to be fulfilled :

A. The histological structure to be analyzed must be isolated without loss or alteration of the chemical components concerned (avoid fixation etc.).

B. The size of the sample to be analyzed must be measured.

C. Analytical quantitative methods of sufficient sensitivity must be available.

D. The possibility of obtaining the data at intracellular level must be available.

E. The nerve cell must be functional after dissection, that is capable of producing regular and sustained impulse activity.

F. The impulse activity must be recorded before, during or after the experiment.

G. The ionic environment must be controlled.

The points E), F) and G) in this list are of particular significance for neurophysiological and neuropharmacological studies. However, in spite of these difficulties, the effort of neurophysiologists, neuropharmacologists

and neurochemists has resulted in two large groups of methods presently available as shown in Table 1.

Among the techniques of histo- and cytochemistry described (Table 2), the quantitative microchemical techniques are obviously those which offer the greatest advantages for their precision and reliability despite the drawback of sometimes being more complicated in their practical use. These techniques are either related to their respective macromethods or have been originally described as such.

As indicated in Table 3 and earlier pointed out by LOWRY & PASSONNEAU (1962), some analytical requirements of a quantitative nature have to be considered before choosing the convenient method. As seen from Table 3 the enzyme activity present in a small cell body or in a $\mu\mu$l sample of cyto- or nucleoplasm can be roughly estimated to 10^{-11} - 10^{-15} moles of product per hour, and the amount of substrate present to be in the order of 10^{-14} - 10^{-18} moles. Which are the analytical techniques able to detect such a small amount of substrate ? If we compare the sensitivity of different techniques, some of them commonly used in our laboratories (Table 4) it can be seen that not only the Warburg technique but even colorimetric techniques are far below the requirements of our analysis. The fluorimetric methods approach the working range for our assay. The cartesian diver and especially the cycling and recycling methods of Lowry are fully satisfactory for our purposes.

Taking into account the above considerations, in the laboratory of neuropharmacology of the Department of Pharmacology of the Karolinska Institute, we have been using in the last few years a series of micromethods which are particularly suitable for biochemical analysis at the cellular level. These methods together with the sensitivity employed by us are reported in Table 5.

Another consideration to be made concerns the choice of the biological material. Processes which take place in one thousandth or less of a second in a membrane of about 80 Å thickness obviously offer great difficulty to chemical analysis. However, progress in a special field is frequently promoted by the availability and the selection of favorable biological material. An evident illustration of this fact is the use of isolated axons of the squid or of crustaceans which proved to be one of the most fruitful materials for neurophysiological investigations.

The discovery of the crustacean stretch receptor organ by Alexandrowitz in 1951 and the recognition of its functions by physiologists in the last 10 years have opened a new possibility, in our opinion, for the attack of neurophysiological problems from the biochemical side. This is particularly so when one reflects that a sensory neuron of these receptors can be regarded as a good model of neurons in general. In fact the crayfish receptor cell

TABLE 1.

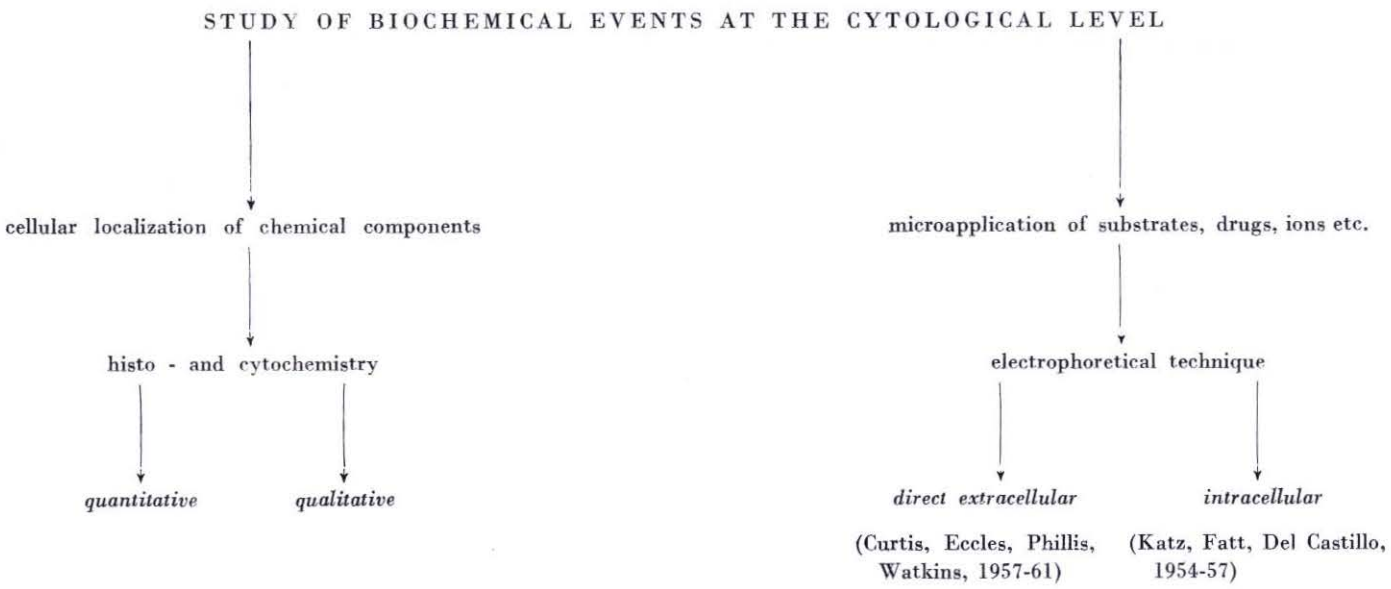

STUDY OF BIOCHEMICAL EVENTS AT THE CYTOLOGICAL LEVEL
cellular localization of chemical components
histo - and cytochemistry
quantitative
qualitative
microapplication of substrates, drugs, ions etc.
electrophoretical technique
direct extracellular
(Curtis, Eccles, Phillis, Watkins, 1957-61)
intracellular
(Katz, Fatt, Del Castillo, 1954-57)

TABLE 2.

HISTOCHEMICAL TECHNIQUES FOR ANALYSIS AT THE CELLULAR LEVEL

Quantitative microchemical technique

I. PREPARATION OF SAMPLES
- A. Centrifugal separations
- B. Chromatographic separations
- C. Microdissection
- D. Microtomy
- E. Freeze-drying

II. MEASUREMENT OF AMOUNT OF SAMPLE
- A. Cell and particle counting
- B. Measurement of volume
- C. Measurement of weight
- D. Measurement of protein
- E. Measurement of RNA and DNA

III. TITRIMETRIC TECHNIQUES

IV. SPECTROPHOTOMETRIC TECHNIQUES ⟷ quantitation of staining reactions; Edström's technique (RNA - DNA)

V. FLAME PHOTOMETRIC TECHNIQUES

VI. FLUORIMETRIC TECHNIQUES → Lowry's microtechnique → DPN and TPN micromodification (cycling)

VII. GASOMETRIC TECHNIQUES → CARTESIAN DIVER of Linderström - Lang → micromodifications

VIII. DILATOMETRIC TECHNIQUES

IX. MICROBIOLOGICAL TECHNIQUES

Qualitative histochemical reactions observed in light microscopy / electron microscopy

TABLE 3.

ANALYTICAL REQUIREMENTS IN BIOCHEMICAL STUDIES WITH ISOLATED NERVE CELLS

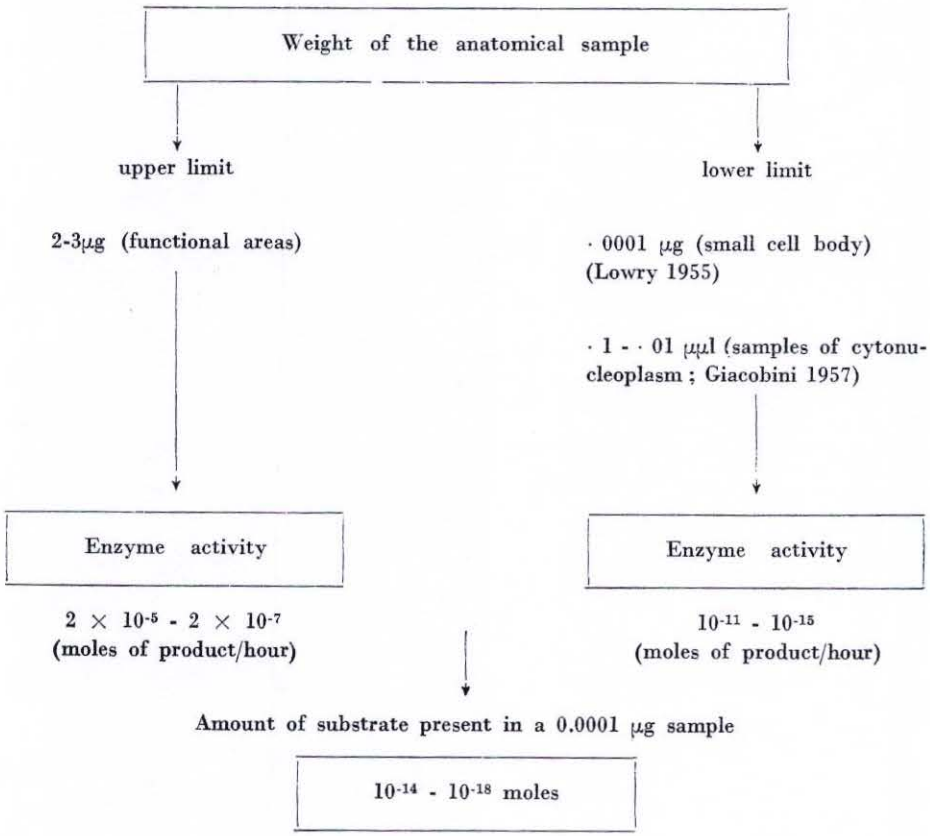
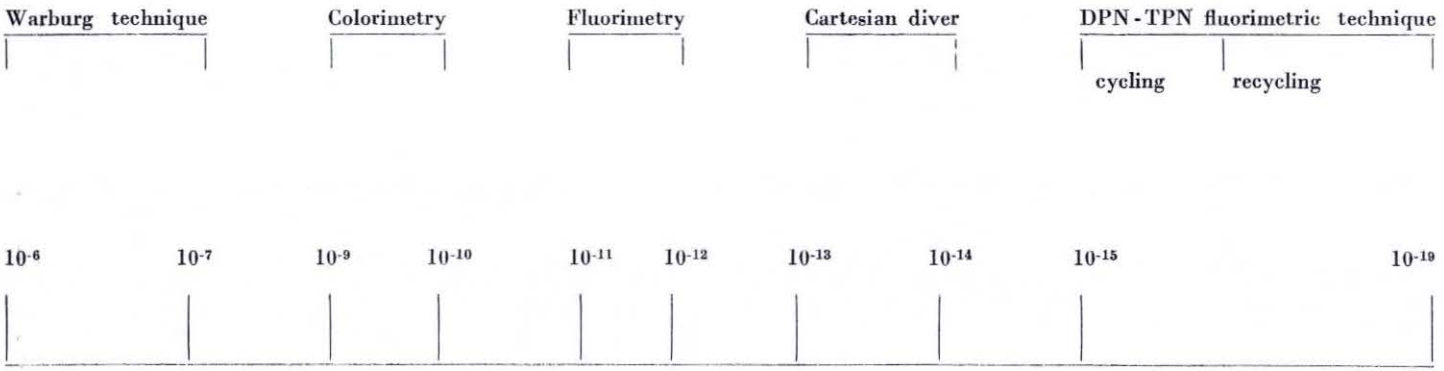


TABLE 4.

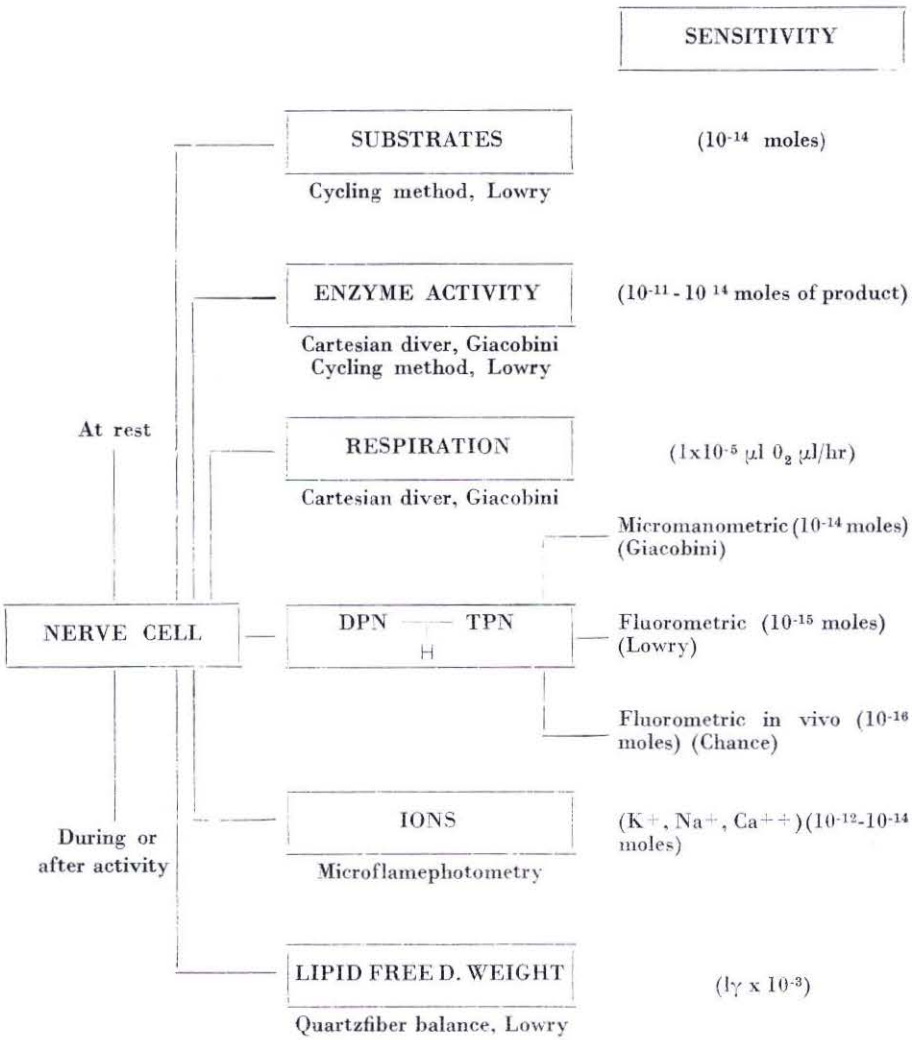
CHOICE OF ANALYTICAL TOOLS IN BIOCHEMICAL STUDIES WITH ISOLATED CELLS



Sensitivity of different techniques in moles of measurable substrate.

TABLE 5.

A SCHEMATIC SURVEY OF METHODS FOR BIOCHEMICAL ANALYSIS AT THE CELLULAR LEVEL TOGETHER WITH THEIR SENSITIVITY EXPRESSED IN MOLES OF PRODUCT PER KG DRY WEIGHT OF SAMPLE



seems to satisfy all the requirements that we have for such a study. *First* it can be easily dissected ; *second* it is capable of producing a regular and sustained impulse activity ; *third* its activity can be recorded both extra- and intracellularly ; and *finally* it can survive for a period of at least 24 hours in the isolated state.

The receptors occur in pairs on both sides of each abdominal segment. Electronmicroscopy (PETERSON & PEPE, 1961) does not reveal any substantial difference in morphology from other types of nerve cells unless the fact that the large dendrites with which the cell is provided are imbedded in a modified muscle fiber and surrounded by connective tissue (CT) as can be seen in Plate I-1 obtained by Dr. P. Peterson. These dendrites (DB in Plate I-1), when stretched give rise to the so called generator potential and contain very peculiar and conspicuous masses of mitochondria. This is of course a region which has a particular relevance not only to the neurophysiologist but also to the biochemist as a potential source of energy.

The standard preparation used in our experiments (GIACOBINI, HANDELMAN & TERZUOLO, 1963) is shown in Plate 1-2 and consists of the neurone cell body of the slowly adapting stretch receptor organ of the crayfish, a segment of axon approximately 600 μ long and a portion of thin muscle bundle, about 400 μ long, in which the dendrites are imbedded. These are the minimal dimensions compatible with survival for most of the preparations (GIACOBINI, HANDELMAN & TERZUOLO, 1963).

The contribution to the measurements by the metabolism of the muscle fiber is estimated in separate experiments (GIACOBINI, HANDELMAN & TERZUOLO, 1965).

Fig. 1 is a schematic representation of the procedure (GIACOBINI *et al.*, 1963). The receptor is prepared by microdissection and its ability to respond to stretch with repetitive impulse activity is tested by means of the forceps applied to the muscle (A). The preparation is reduced to the minimal standard dimension (see also diagram in Plate I-2), and its functional integrity can then be tested by recording the impulse activity from the axon with a 20 μ wire electrode EL (B). Although no forceps can be applied to the muscle to produce stretch after the preparation has been cut to the stated minimal dimensions, stretch can still be applied when the axon is raised into the air because of the surface tension of the solution. The impulse activity is displayed by an oscilloscope and audio monitored (B). In these conditions the cell can still be stimulated by varying the K^+ and Ca^{++} concentrations in the medium (GIACOBINI, HANDELMAN & TERZUOLO, 1963). A rise of the K^+ concentration of less than twofold produces a spontaneous firing of the cell which can last several hours. The frequency of the impulse activity is recorded by a digital computer. To perform the microgasometric measurements, the preparation is introduced by suction into a capillary diver together with a small amount

(about 0.5 μl) of the appropriate medium (C) and placed at the desidered position (D). The microdiver is subsequently sealed and, after a period of equilibration, measurements are made for at least one hour (E). The diver is then opened and the functional integrity of the cell is tested as in B (F). Steps C to F can be repeated several times without apparent damages to

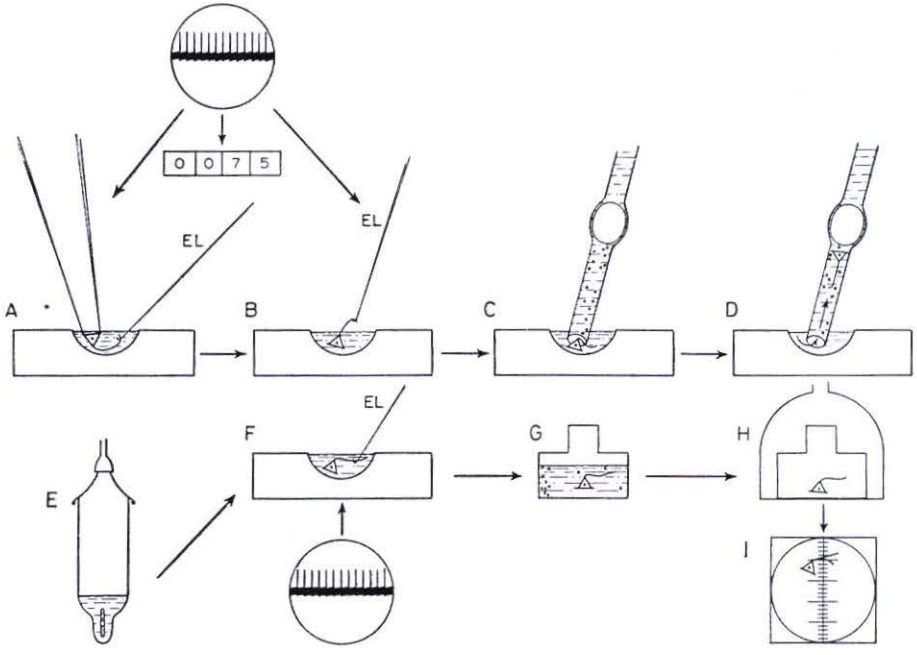


Fig. 1. — Simplified diagram of the experimental procedure for measurement of oxygen uptake and enzyme activity in single nerve cell preparations at rest and during impulse activity. For description see text.

the cell. In the next step (G) the preparation is dehydrated and the lipids extracted with alcohol (95 per cent) and dioxane. After dehydration, the alcohol is boiled off at room temperature under high vacuum (H) and the preparation is weighed (I) with a quartz fiber microbalance of the type described by Lowry (1941).

A single nerve cell preparation weighs (lipid-free dry weight) between 0.1 μg-0.01 μg. The oxygen uptake by the preparation in resting conditions and in absence of any added substrate was 4.18×10^{-4} μl 0_2 cell/hour ($p = 0.05$, range = 3.51-4.85) (Giacobini *et al.*, 1963).

These data can be compared with the average neuronal oxygen consumption of the brain cortex neurone which was estimated by Kety & Schmidt (1948) to be about 3×10^{-4} μl 0_2 cell/hour *in homo*, by Elliott & Heller (1957) 0.63×10^{-4} μl 0_2 cell/hour in the cat, by Korey & Orchen (1959)

1.36×10^{-4} µl 0_2 cell/hour in the lamb and finally by EPSTEIN & O'CONNOR (1965) 10.2×10^{-4} µl 0_2 cell/hour in the cat.

These estimations are however difficult to correlate to our results in the living neurone since they were made on material from fresh or frozen-dried slices of brain or from cell fractions.

Fig. 2 shows the marked increase in respiratory rate observed after different and progressively increasing frequency of impulse activity. It can be calculated that an increase in oxygen consumption of this magnitude may correspond to a heat production of 25 µcal/g/impulse.

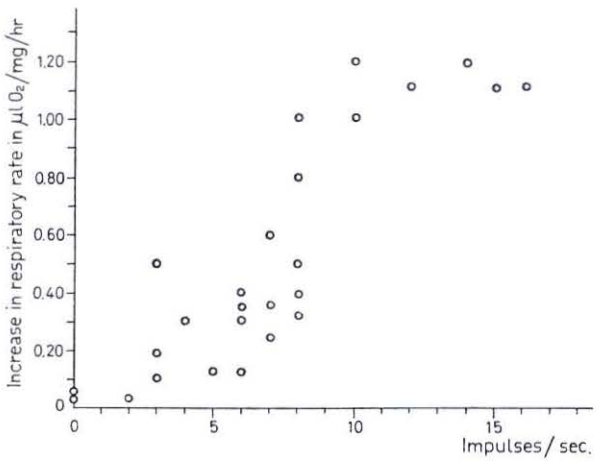


Fig. 2. — Relationship between respiratory activity and frequency of impulse activity in single crustacean nerve cell preparations.

ABBOTT, HILL & HOWARTH (1958) estimated a heat production of 2 µcal/g/impulse in the nerves of the spider crab.

For the reasons which were outlined in the introduction of this paper, our efforts were directed towards the determination of the effects of an altered ionic content of the external medium (GIACOBINI *et al.*, 1965). Such alterations exert well known effects upon the electrical properties of the cell membrane. (EDWARDS, TERZUOLO & WASHIZU, 1963). Could they also involve the general metabolism of the cell and in particular the O_2 consumption ? It must be noted in this respect that the nerve cell of the stretch receptor organ is possibly the only sensory cell where these membrane properties can be studied when the external ionic environment is controlled. The effect of varying concentrations of 5 different ions (K^+, Ca^{++}, Mg^{++}, Na^+, Cl^-) or the total omission of one of these from the solution was therefore investigated (GIACOBINI *et al.*, 1965).

The oxygen uptake in Ca^{++} free solution was found to be markedly reduced (see Fig. 3 and 5). In this situation it can be assumed that the cell is electrically inexcitable and both the resting membrane potential and the membrane resistance are greatly reduced (EDWARDS *et al.*, 1963). The respi-

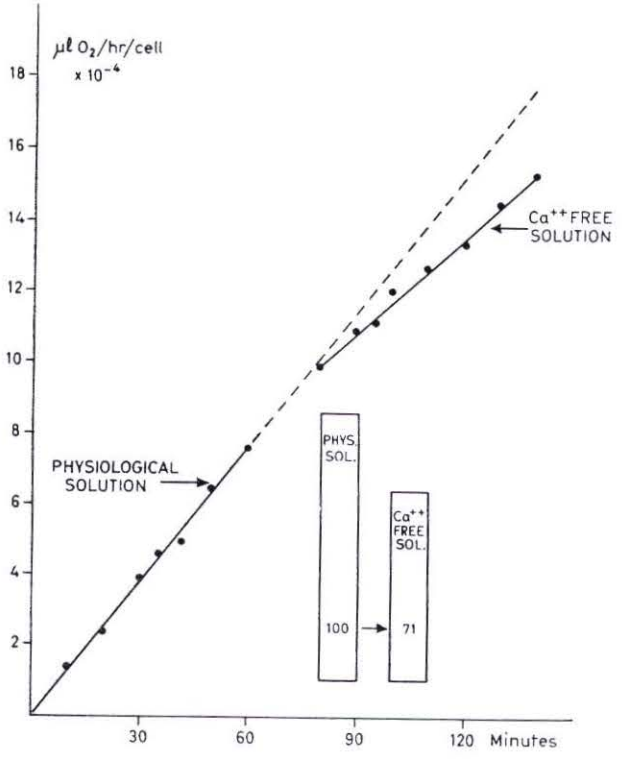


Fig. 3. — Oxygen uptake of a single crustacean nerve cell preparation in Van Harreveld physiological solution and in Ca^{++} free solution. Bar graph shows the final relationship in per cent.

ration experiment shown in Fig. 4 consists of three different steps. The oxygen consumption expressed in µl O_2/cell/time unit is again shown by the slope of the curves. In A the cell is firing 7 impulses per second (the K^+ concentration has been increased almost 2-fold), in B the cell is brought back to the physiological solution and no spontaneous impulse activity is present. The slope indicates a decrease in oxygen consumption. Finally in C the K^+ concentration has been increased 10 times and the slope of the curve indicates a further decrease of the oxygen consumption. In the above condition the action potential is absent and the membrane resistance and the resting potential reach very low values (EDWARDS *et al.*, 1963).

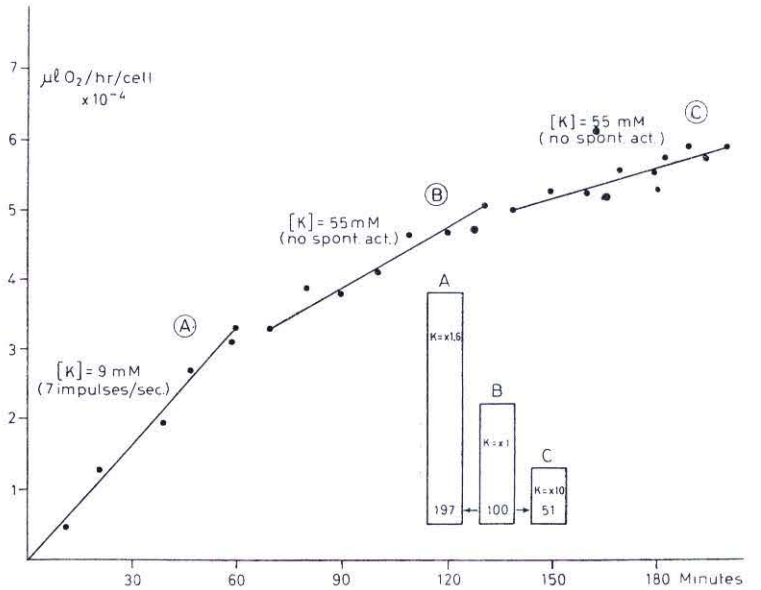


Fig. 4. — Oxygen uptake in a single crustacean nerve cell preparation in Van Harreveld physiological solution (B) and in 1.6 (A) and 10 times (C) K^+ solutions. Bar graph shows the final relationship in per cent.

In Fig. 5 the oxygen uptake of a single nerve cell preparation in various electrolyte solutions is expressed as per cent of uptake in the physiological solution. It can be seen that when Ca^{++} is completely removed, the respiration of the nerve cell is reduced to 61 per cent of the normal; in a high K^+ solution (12-fold normal) this is reduced to 52.5 per cent; in Na^+ free solution to 54 per cent; and in Cl^- free solution the oxygen consumption is practically the same as in the physiological solution (GIACOBINI, 1964 ; GIACOBINI *et al.*, 1965).

The effect of nineteen metabolic and ion transport inhibitors acting at various enzymatic levels within several metabolic pathways was investigated by GIACOBINI (1965) upon a single neurone preparation of the crayfish stretch receptor organ. Both the function of the nerve cell as judged by its impulse activity and its metabolism expressed by oxygen uptake were studied. Each of the inhibitors was tried at various concentrations and pH's. The effect of the respiratory inhibitors, especially rotenone and antimycin A, suggests a coupling of respiration and impulse activity in the crustacean nerve cell. The marked effect of the glycolytic inhibitors and of Na-malonate strongly suggests a link between glycolysis and Krebs' cycle on the one side and maintenance of impulse activity on the other. This view is also sup-

ported by the effect of glucose restoring activity after the blocks caused by several inhibitors. Ouabain and digoxin inhibit both impulse activity and oxygen uptake. In general, respiration seems to be less sensitive to inhibitors than is the impulse activity, indicating the presence of a safety factor for respiratory processes.

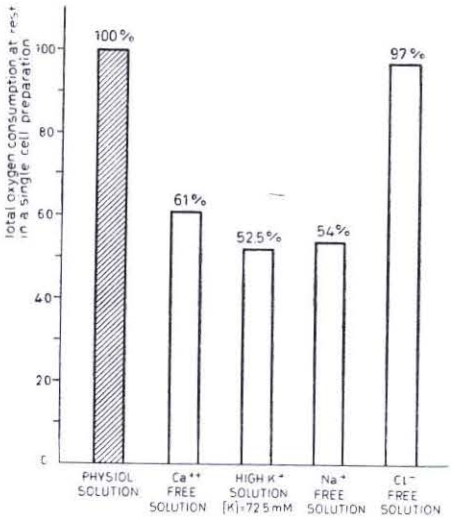


Fig. 5. — Oxygen uptake in single nerve cell preparations in various electrolyte solutions expressed as per cent of uptake in the physiological solution. Each percentage value represents the median value of ten determinations.

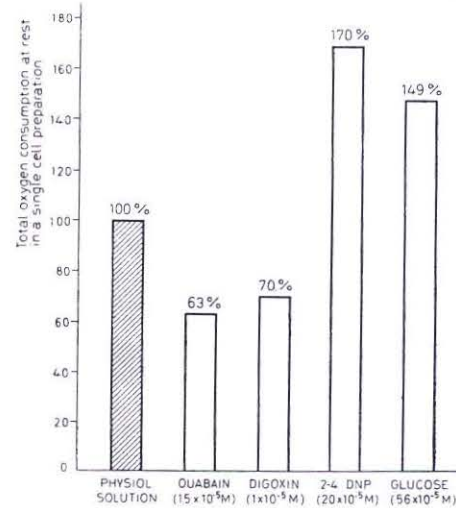


Fig. 6. — Oxygen uptake of single cell preparations in various solutions in the presence of metabolic inhibitors and glucose expressed as per cent of uptake in the physiological solution. Each percentage value represents the median value of ten determinations.

In Fig. 6 the oxygen uptake of single nerve cell preparations in the presence of various metabolic inhibitors or glucose is compared to that in physiological solution. A marked reduction of respiration is noticed in the presence of ouabain and digoxin, respectively (63 and 70 per cent), whereas in the presence of 2,4-dinitrophenol the respiration is increased by 170 per cent (GIACOBINI *et al.*, 1965).

As a direct consequence of our results with metabolic inhibitors indicating glycolysis as a probable source of substrates for oxidative processes linked to impulse activity, GRASSO & GIACOBINI (1965) decided to investigate the level of several glycolytic intermediates, both at rest and after prolonged physiological stimulation, using the newly described biochemical technique of LOWRY *et al.* (1964). Phosphate compounds and pyridine nucleotides were also determined using the same method. The technical procedure employed and the cycling method of LOWRY *et al.* (1964) are schematically illustrated

in Fig. 7 and Fig. 8. In the first step in Fig. 8 hexokinase and glucose-6-phosphate dehydrogenase, in the second glutamate dehydrogenase and glucose-6-phosphate dehydrogenase and in the third step 6-phosphogluconic dehydrogenase were present.

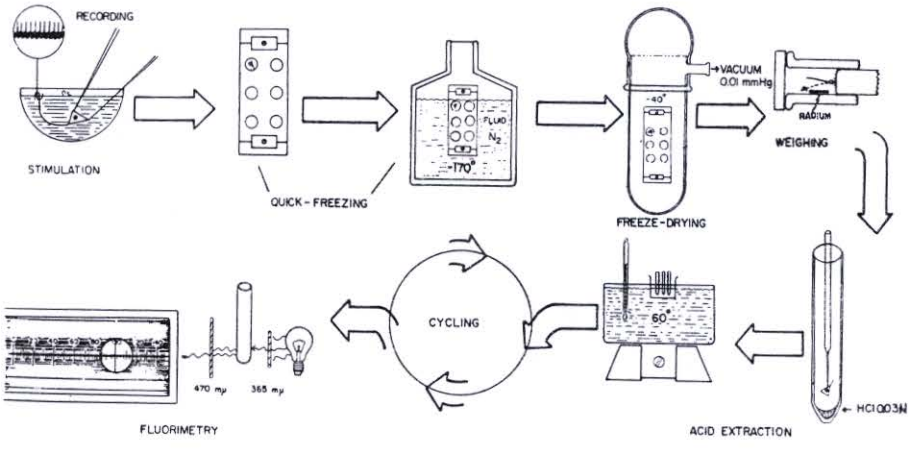


Fig. 7. — Schematic description of the analytical procedure used for determination of metabolic intermediates, pyridine nucleotides and phosphate compounds in single crustacean nerve cell preparations. See text.

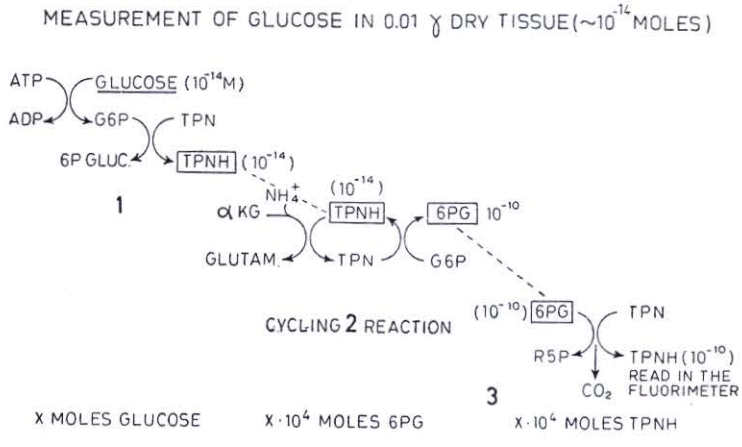


Fig. 8. — Schematic illustration of the cycling method described by Lowry *et al.* Determination of glucose in a 1.01 μg sample of tissue. In the first step hexokinase and glucose-6-phosphate dehydrogenase, in the second glutamate dehydrogenase and glucose-6-phosphate dehydrogenase and in the third step 6-phosphogluconic dehydrogenase are present.

The dynamic variations in the substrate level after activity can be followed in Fig. 9 where the per cent variations from the resting state are reported. The glycogen decreased consistently with the lactate increase during impulse activity. ATP showed a continuous decrease during prolonged stimulation while ADP and Pi showed opposite patterns. After stimulation, the ratio TPN/TPHN fell by more than 50 per cent. This was due to a decrease of TPN. Similarly, the DPN/DPNH ratio fell by 50 per cent, but in this case it was due to a decrease of DPN and a simultaneous increase of DPNH. The results strongly suggest the involvement of glycolysis in the mechanism maintaining impulse activity in this neurone. Impulse activity in the crustacean nerve cell is linked to an energy requiring system ; this energy is furnished by ATP splitting and glycolysis directly or through the arginine phosphate step supporting the ATP resynthesis.

The effect of seven intermediates of the tricarboxylic acid cycle, of pyruvate and glutamate was studied by Marchisio (Marchisio & Giacobini,

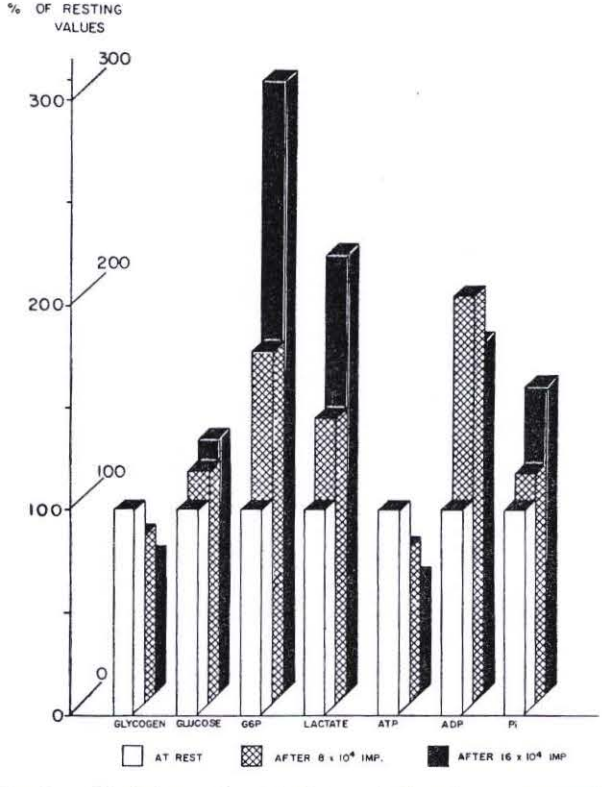


Fig. 9. — Variations of metabolites and phosphate compounds in the slowly adapting stretch receptor cell after [8×10^4 and 16×10^4] impulses. Average values from several experiments.

1965) in our laboratory, on the impulse activity and oxygen uptake of the slowly adapting cell of the stretch receptor organ of crayfish. Citrate, isocitrate, α-ketoglutarate and succinate showed a strong excitatory action, fumarate, malate, oxaloacetate and pyruvate had no action while glutamate had an inhibitory action on the impulse activity (see Fig. 10). The most effective substrate tested was citrate which also increased respiratory sevenfold. Isocitrate showed a lower effect (2.2-fold) as did ketoglutarate (4-fold),

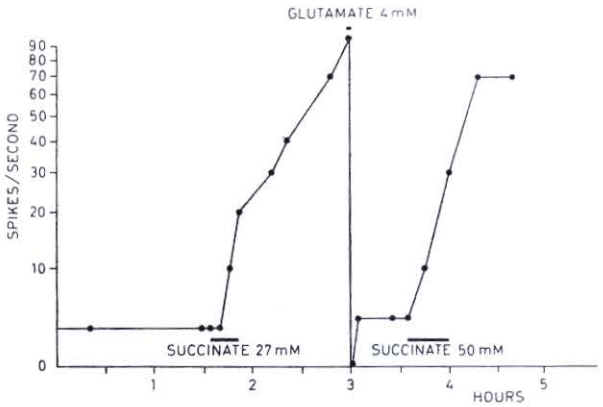


Fig. 10. — A typical experiment showing the effect of 27 mM succinate and 4 mM glutamate on the impulse activity of an isolated slowly adapting stretch receptor cell.

succinate (2.8-fold) and malate (3-fold). Fumarate, pyruvate and glutamate did not have any effect on the respiratory activity. The only substrate showing an inhibitory effect on oxygen uptake was oxaloacetate. Glucose, tested for comparison, also showed stimulatory effect (2.4 fold) on respiration. The authors are inclined to believe that the effect on the impulse activity and respiration exerted by these compounds is confined to the nerve cell membrane. The opposite modes of action of malate and oxalaocetate on respiration, which did not affect impulse activity, are however more difficult to explain in terms of a pure membrane mechanism.

The effect of the respiratory inhibitors, especially rotenone and Antimycin A, as previously commented, suggests a coupling of respiration and impulse activity in the crustacean nerve cell. This is also in agreement with the reported presence of respiratory enzymes and cytochromes (MUNRO, 1955) in crustacea, and with the findings of TERZUOLO *et al.* (1964) and BONEWELL & GIACOBINI (unpublished results), that the activity of oxidative enzymes is increased during impulse activity.

In order to decide whether some of our results, especially those obtained with inhibitors, may be related to active transport processes it has been

necessary to determine Na and K in cells treated with different inhibitors or in conditions of modified ionic environment. A modified microflamephotometric method for the simultaneous determination of sodium and potassium has therefore been developed (GIACOBINI, 1965). The sensitivity of this method is in the order of 10^{-12} - 10^{-14} for Na and K respectively. A single cell preparation has been found to contain $1.74 \pm 0.1 \times 10^{-10}$ moles K (mean of eight experiments $\pm$ S.D.) corresponding to about 150 mEq. Measurable variations of ion concentrations in the presence of inhibitors and modified ionic environment have been detected.

Summarizing our results, it appears that the prolonged physiological stimulation of a single nerve cell preparation initiates a sequence of biochemical events now readily measurable at this level. The parameters which can actually be measured are the following : oxygen consumption, enzyme activity, the level of several compounds including glycolytic and citric acid cycle intermediates, oxidized and reduced pyridine nucleotides, phosphate compounds and finally ions. Most of these parameters have already been measured and a part of the results have been reported in this paper. Our results emphasize the importance of glycogen as a source of energy in the crustacean nerve cell related to impulse activity and suggest a general scheme of metabolic control similar to that reported by MAITRA *et al.* (1964) for the electric organ. In such a scheme, modified in Fig. 11, the presence of the enzyme ATPase in the membrane of the nerve cell has to be postulated, the energy store is represented by arginine phosphate and the source of energy the glycolytic process itself.

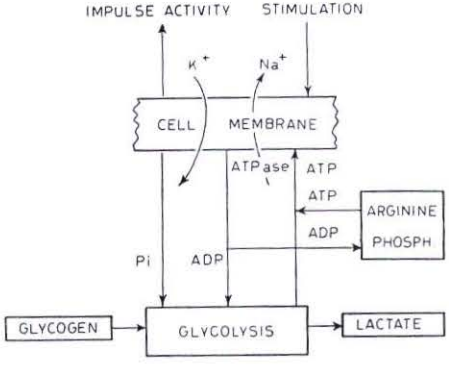


Fig. 11. — A general scheme of neurophysiological and metabolic correlations in the slowly adapting cell of the stretch receptor of crustacea. See text.

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Summary. — The combination of sensitive micromethods and neurophysiological techniques offers the possibility of investigating biochemical processes connected with the function of nerve cells. Some criteria for selecting useful methods for analysis at the cellular level are discussed. The parameters which can actually be measured are the following : oxygen consumption, enzyme activity, the level of several metabolites including glycolytic and citric acid cycle intermediates, oxidized and reduced pyridine nucleotides, phosphate compounds and ions. Most of these parameters have already been measured in our laboratory and a part of the results are reported in this paper. Our results show that prolonged physiological stimulation of single nerve cell preparations initiates a sequence of biochemical events now readily measurable and emphasize the importance of glycogen as a source of energy in the crustacean nerve cell.

Riassunto. — La combinazione di micrometodi altamente sensibili con tecniche neurofisiologiche offre la possibilità di analizzare a livello cellulare variazioni di carattere biochimico connesse con la funzionalità della cellula nervosa. Alcuni criteri per la selezione del tipo di microtecniche da usarsi vengono discussi nella prima parte di questo lavoro. I parametri già accessibili alle nostre tecniche sono i seguenti : respirazione cellulare, determinazione di attività enzimatica, determinazione quantitativa di substrati e tra questi di metaboliti della glicolisi e del ciclo di Krebs, piridino - nucleotidi ossidati e ridotti, fosfati organici e ioni. La maggior parte di tali parametri è stata misurata nel nostro laboratorio in singole cellule nervose a riposo e durante attività ed alcuni risultati vengono qui riportati e commentati. Tali risultati dimostrano che la stimolazione fisiologica prolungata inizia una serie di variazioni biochimiche attualmente misurabili coi nostri metodi e mette in rilievo il processo glicolitico come sorgente di energia collegata all'attività della cellula nervosa.

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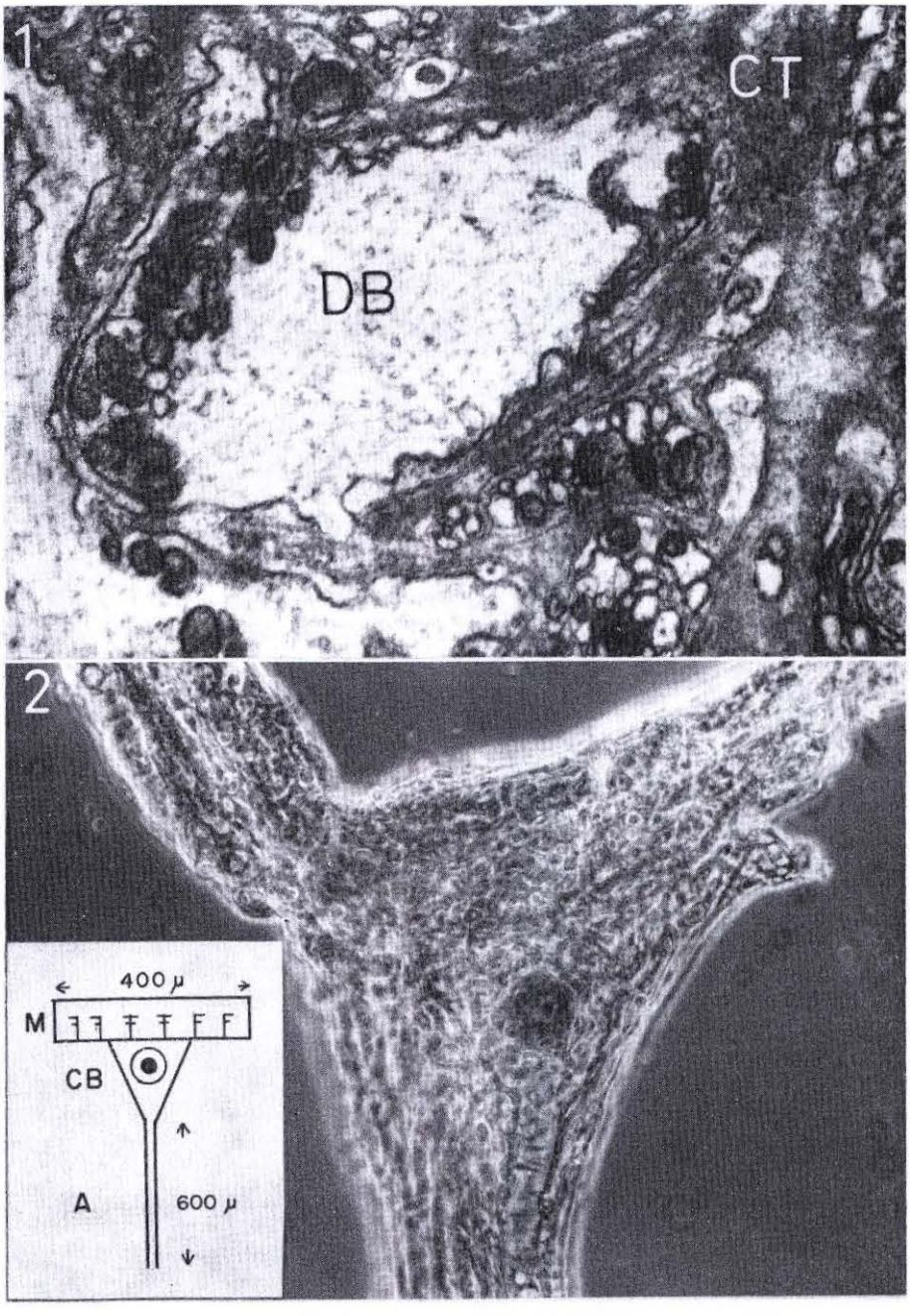
Plate I. - 1. Electron micrograph showing a dendritic branch (DB) of the stretch receptor cell of crayfish. Notice heavy cumulations of mitochondria inside the dendryte and the connective tissue (CT) outside it. Magn. about 32.000. (The picture was taken by Dr. P. Peterson).

2. Photomicrograph of unstained living slowly adapting receptor cell of crayfish. The thin muscle fiber (upper part), the nucleus with the nucleolus in the center of the cell body and the axon (lower right) are visible. The glial tissue surrounding the cell body has not been teased out. In the corner : simplified diagram of the elements of the standard preparation with measurements. M = muscle fiber ; CB = cell body ; A = axon. See text for description.


1
CT
DB
2
400 μ
M
CB
A
600 μ