amine, homocystine, cystine hydantoin, homocystine hydantoin, diformylhomocystine.

2. It has been found that positively charged compounds containing disulphide bonds react much faster with SO_3^{2-} ions than do disulphides with no net charge.

3. The pK of the thiol group of *N*-formylhomocystine was calculated from equilibrium data. This group has a heat of ionization of approximately + 6000 calories.

4. The influence of chemical environment on the reactivity of protein functional groups is discussed.

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Changes in the Nucleic Acid Content of Ganglion Cells during Chromatolysis and after Stimulation

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This paper is concerned with the changes in total nucleic acid and deoxyribonucleic acid (DNA) in the superior cervical ganglion of the rabbit after section of the postganglionic fibres or stimulation of the preganglionic fibres.

In peripheral nerve it has been shown (Logan, Mannell & Rossiter, 1952*b*) that after section of the nerve the total nucleic acid increases in the distal stump to a maximum at about the sixteenth day after section and that this rise in total nucleic acid is due largely to a marked increase in the ribonucleic acid (RNA) fraction. Similar, but less marked, changes can be detected during the first 48–96 hr. after nerve section, and furthermore it has been shown that intense stimulation of the normal nerve trunk produces a reduction in the DNA fraction while leaving the total nucleic acid unchanged (Causey & Stratmann, 1956). Hydén (1943), using Caspersson's ultraviolet-absorption technique, showed a decrease in the RNA content

of the anterior-horn cells of the spinal cord after intense motor activity.

It was apparent that if the more simple chemical method of nucleic acid estimation was to be applied to nerve-cell bodies, a more uniform and discrete group of cells than those used by Hydén would have to be employed. The cells of the superior cervical ganglion were used. The proportion of nerve cells to supporting tissues and fibres is high, and it is possible to subject the cells both to section of the nerve processes by cutting the postganglionic fibres and to excitation by stimulation of the preganglionic trunk.

EXPERIMENTAL

Rabbits of about 2.5 kg. body weight were used throughout; no selection was made for breed or sex. All survival operations were carried out under Nembutal (pentobarbitone sodium, B.P.) and ether anaesthesia. The specimens were dissected out under urethane anaesthesia. The superior

cervical ganglia were dissected out carefully, freed of fat and fibrous connective tissue, and weighed wet. After section of the postganglionic fibres there is a considerable increase in the wet weight of the ganglion; therefore the wet weight of the normal ganglion of the opposite side was used as a measure of the original weight of the ganglion subjected to operation, and the results have been calculated in these terms. A similar procedure was also used for the stimulated ganglia which had been in contact with liquid paraffin. To estimate the error of this procedure a number of ganglia from opposite sides of normal rabbits were weighed, and although individual pairs varied considerably in weight the differences in the wet weight between pairs of ganglia amounted to only 0.24 ± 0.048 mg. for fourteen pairs of ganglia. The average wet weight of a ganglion was about 10 mg.

Chromatolysis of the nerve cells was produced by section of all the cephalic branches of the ganglion close to the body of the ganglion. The animals were killed 7 or 14 days later. Excitation of the normal ganglion was produced by stimulation of the cervical sympathetic chain at a frequency of 300 cyc./sec. for a period of 3 hr., at a stimulus strength just supramaximal as judged by the compound action potential observed on an oscilloscope. The nerve and stimulating electrodes were immersed in liquid paraffin.

The ganglia were analysed for total nucleic acid and DNA. The method of analysis was essentially similar to that described by Logan, Mannell & Rossiter (1952a). The ganglia, cleaned of any adherent fatty and connective tissue, were weighed wet and homogenized in ice-cold 10% (w/v) trichloroacetic acid in a test-tube homogenizer. Acid-soluble P and lipid were removed from the homogenate by treatment with further quantities of trichloroacetic acid, ethanol and ether-ethanol according to the method of Schneider (1945). One series of samples was then extracted for 15 min. at 90° with 5% (w/v) trichloroacetic acid. This was used for determination of total nucleic acid by ultraviolet absorption. The other series of samples, after treatment as above for removal of acid-soluble P and lipid, was hydrolysed for 18 hr. at 37° in *N*-KOH. DNA was precipitated by the addition of 5*N*-HCl and 5% (w/v) trichloroacetic acid to the hydrolysate. The DNA was removed by centrifuging. The precipitated DNA was then extracted with 5% (w/v) trichloroacetic acid for 15 min. at 90° and estimated by ultraviolet absorption.

The final estimation of total nucleic acid and DNA was made by determination of the optical densities of the solutions at a wavelength of 268.5 mμ. with a Hilger Uvispek spectrophotometer.

Trichloroacetic acid itself absorbs appreciably at this wavelength, therefore a blank consisting of 5% (w/v) tri-

chloroacetic acid was used. As trichloroacetic acid tends to be broken down during heating at 90°, it was necessary for the samples and blanks to be heated for exactly the same length of time. The optical density of 5% (w/v) trichloroacetic acid after heating for 15 min. at 90° was found to be approximately 0.150 at 268.5 mμ. in a 1 cm. cell.

The method was standardized by the use of stock solutions of DNA and RNA obtained commercially from Harrington Bros. The results are expressed in terms of mg. of nucleic acid P/100 g. wet wt. of tissue. The standard curves relating absorption and mg. of nucleic acid P were constructed by use of the stock solutions. The absorption characteristics and P content of these standards are shown in Table 1.

Since the amount of superior cervical ganglion tissue available was small it was not possible to estimate both total nucleic acid and DNA on the one sample from a single animal. Hence two series of experiments were carried out, in one of which total nucleic acid was estimated, and in the other DNA. Therefore a direct calculation of the RNA content (total nucleic acid - DNA) in each case, as was done previously (Logan *et al.* 1952a, b; Causey & Stratmann, 1956), was not possible.

A number of estimations of the water content of the tibial nerves of adult and immature rabbits has been carried out by weighing pieces of nerve before and after heating to constant weight in an oven at 110°.

RESULTS

The results are summarized in Tables 2-5. In the normal superior cervical ganglion (Table 2a) there is about 44 mg. of nucleic acid P/100 g. wet wt. of tissue, and of this 13 mg. is represented by the DNA fraction. Seven days after section of the postganglionic fibres (Table 2b) total nucleic acid was found to have doubled, and by the 14th day to have reached three times the original amount in the ganglion (130 mg.). At the same time the DNA fraction had also risen, so that at both the 7th and 14th day after section the DNA was about four times that originally present (50 mg.). Whilst direct calculation of the difference, total nucleic acid minus DNA, is impossible for the reasons given above, the difference between the means of the two groups of estimations appears to rise very sharply up to about 80 mg. of nucleic acid P/100 g. wet wt. of tissue by the 14th day. The fraction concerned in this rise would be, presumably, RNA.

Table 1. *Phosphorus content and absorption characteristics of standard preparations of DNA and RNA*

ϵ (P), the atomic extinction coefficient with respect to P, is defined: ϵ (P) = $\frac{30 \cdot 98 E}{c l}$, where $E = \log I_0/I$, c = concentration of P in g./l., and l = thickness of absorbing solution in cm. (Chargaff & Zamenhof, 1948. TCA: trichloroacetic acid.)

DNA	λ (mμ.)	ϵ (P)	P content (%)
In water	259 ($\lambda_{\max.}$)	8980	6.9
After heating for 15 min. in 5% TCA	268.5	9820	—
RNA			
In water	258 ($\lambda_{\max.}$)	9450	8.5
After heating for 15 min. in 5% TCA	268.5	9820	—

Table 2. *Nucleic acid content of the superior cervical ganglion of the rabbit*

(a) From normal rabbits, (b) from rabbits in which all the cephalic branches of the ganglion were sectioned 7 days prior to biopsy, and (c) from rabbits in which all the cephalic branches of the ganglion were sectioned 14 days prior to biopsy. For the normals the results are expressed in mg. of nucleic acid P/100 g. wet wt. of ganglion. For operated ganglia results are expressed in terms of mg. of nucleic acid P/100 g. wet wt. of the unoperated ganglion from the opposite side. Number of estimations is given in parentheses after each result. Results are given as mean $\pm$ standard error of mean.

	Total nucleic acid	DNA
a	43.7 $\pm$ 2.20 (29)	13.0 $\pm$ 0.60 (39)
b	90.4 $\pm$ 6.47 (8)	48.5 $\pm$ 5.54 (10)
c	128.7 $\pm$ 3.84 (9)	51.0 $\pm$ 2.22 (9)

Table 3. *Nucleic acid content of the superior cervical ganglion of the rabbit*

Ganglia were (a) excited by stimulating the cervical sympathetic chain in the neck for 3 hr. at a frequency of 300 cyc./sec. under liquid paraffin, and (b) soaked in paraffin for a similar period and under exactly the same conditions but without stimulation. The results are expressed in terms of mg. of nucleic acid P/100 g. wet wt. of ganglion taken from the untreated side. Number of estimations is given in parentheses after each result.

	Total nucleic acid	DNA
a	43.3 $\pm$ 1.95 (10)	32.8 $\pm$ 3.65 (9)
b	—	11.8 $\pm$ 0.99 (9)

Table 4. *Mean nucleic acid content and its standard error of (a) the superior cervical ganglion of rabbits 1-2 days old, (b) the tibial nerve of rabbits 1-2 days old and (c) the connective-tissue sheath of the tibial nerve of adult rabbits*

Results are expressed in terms of mg. of nucleic acid P/100 g. wet wt. of fresh tissue. Number of estimations is given in parentheses after each result.

	Total nucleic acid	DNA
a	81.4 $\pm$ 2.67 (7)	18.6 $\pm$ 2.40 (5)
b	49.2 $\pm$ 3.46 (14)	17.6 $\pm$ 1.17 (16)
c	17.1 $\pm$ 1.70 (7)	8.2 $\pm$ 0.98 (6)

Table 5. *Mean water content and its standard error of (a) the tibial nerve of adult rabbits and (b) the tibial nerve of rabbits 1-2 days old*

Results are expressed as the percentage of water in the wet nerve. Number of estimations is given in parentheses after each result.

	Water content (%)
a	67.8 $\pm$ 1.64 (8)
b	75.4 $\pm$ 1.17 (8)

When the preganglionic fibres to the ganglion were subjected to a long period of fast repetitive stimulation (Table 3a) no change was found in the total nucleic acid content of the ganglion, but a sharp rise in the DNA was observed, the content increasing nearly three times to some 33 mg. of DNA P/100 g. wet wt. of tissue. Control estimations (Table 3b) were made on ganglia left *in situ* and surrounded with liquid paraffin for 3 hr. without stimulation. There was no significant difference in the DNA content of these ganglia from the normals.

In an attempt to assess the significance of these marked changes that occur in total nucleic acid and DNA on degeneration and stimulation, estimations of the total nucleic acid and DNA content were made in (1) very immature rabbits (1-2 days old), and (2) the fibrous-tissue sheath of the tibial nerve of adult rabbits. The results of these estimations are shown in Table 4. With immature rabbits, at least four superior cervical ganglia had to be pooled in order to obtain enough tissue for analysis. The tibial nerves, however, were of sufficient size to permit analysis to be carried out on each nerve.

It will be seen that both the superior cervical ganglia and the tibial nerves of immature rabbits show high values of both total nucleic acid and DNA as compared with the corresponding tissues of the adult rabbit. The superior cervical ganglia have a total nucleic acid content twice that found in the adult, although the DNA was not much higher. The tibial nerves show both total nucleic acid and DNA levels approximately seven times the adult levels (Causey & Stratmann, 1956).

The fibrous-tissue sheath estimations were carried out on epineural sheath stripped from adult tibial nerves. The results of these estimations are also shown in Table 4. These estimations show high levels of both total nucleic acid and DNA, of the same order of magnitude as that found in adult whole tibial nerve.

The results of estimations of water content of the tibial nerves of adult and immature rabbits are shown in Table 5. It will be seen that there is very little difference in water content between the two groups of nerves, the tibial nerves of young rabbits (1-2 days old) having, as would be expected, a slightly higher water content than adult nerve.

DISCUSSION

The great changes in the nucleic acids of the superior cervical ganglion reported in this paper provide further evidence of the lability of these components in nervous tissue (Causey & Stratmann, 1956). The nucleic acid content, expressed in terms of wet weight of tissue, in superior cervical ganglia and nerve of young rabbits (1-2 days old), has been found to be much greater than that found in adults.

This difference cannot be due to differences in water content between adult and immature tissue, as is shown in Table 5. Nor does it seem likely that, in tibial nerves, differences in amount of epineural sheath could be responsible, for it has been found that sheath taken from adult nerves contains considerable quantities of nucleic acids. It seems most likely that this higher level of nucleic acid, particularly in the RNA fraction, is associated with growth processes in the young animal.

Total nucleic acid is known to increase in the peripheral stump during degeneration of nerve and closely follows proliferation of Schwann cells (Logan *et al.* 1952*b*), but it is uncertain how far or what proportion of the increase during the first 48–96 hr. is due to changes in Schwann cells (Causey & Stratmann, 1956). The increase of nucleic acid content in the ganglia after severance of the axons, on the other hand, is likely to be due mainly to changes in the nerve-cell bodies, and is, presumably, linked with the process known to histologists as chromatolysis (Gersh & Bodian, 1943; Hydén, 1943). The changes reported here show at first an increase involving both DNA and RNA, and later mainly RNA, perhaps in association with the synthesis of new axoplasm.

The changes in nucleic acids as a result of stimulation are more difficult to evaluate. In a peripheral nerve trunk, after stimulation, the total nucleic acid content remains constant but RNA was found to increase while DNA falls (Causey & Stratmann, 1956). However, preganglionic excitation of the superior cervical ganglion leads to an increase in DNA and a fall in RNA without change in the total nucleic acid content of the cells. The cellular processes involved in these changes remain obscure, but it is clear that the nucleic acids of both cell body and nerve fibre play an intimate part in functional activities. The opposing trends in the ganglion and the peripheral nerve trunk could perhaps be attributed to the necessity of re-establishing a steady state.

SUMMARY

1. Total nucleic acid and deoxyribonucleic acid (DNA) have been determined in the superior cervical ganglion of the rabbit after section of the postganglionic fibres and after stimulation of the preganglionic fibres.

2. The normal levels of total nucleic acid and DNA in the superior cervical ganglion were 44 mg. of nucleic acid P/100 g. wet wt. of tissue and 13 mg. of nucleic acid P/100 g. wet wt. of tissue, respectively.

3. Total nucleic acid and DNA both increased after section of the postganglionic fibres. At 14 days after section the level of total nucleic acid was 130 mg. and of DNA 50 mg. The main increase is presumably in the ribonucleic acid fraction.

4. After stimulation of the preganglionic fibres total nucleic acid remained unchanged but DNA increased markedly.

5. Estimations of total nucleic acid and DNA content of the superior cervical ganglion and tibial nerve of very immature rabbits are also reported.

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