

drying of the secretion, and he thought it more probably due to stimulation of the cilia. Thus it is possible to observe how easily the theory of the action slips from what is rational and has some foundation to simple speculation.

Certainly neither the bacteriostatic action nor the stimulation of cilia can explain the efficacy of oral chlorophyll preparations in reducing perspiration and other body odours, which is widely claimed. Thus le Vann⁶ has stated that in a mental institution the commonest reason for resignation of staff was the smell from incontinent patients. The patients were separated into two wards and given tablets of a chlorophyll derivative, all antiseptics being removed. There was a striking reduction in the smell. Must we then suppose that chlorophyll prevents incontinence? Reports of this kind, and those relating to reduction of underarm odour in industrial workers and college athletes,^{7, 8} are very difficult to understand and to credit. This is particularly so when these authors make tests to show that "raw chlorophyll" and a "copper salt of chlorophyll" are insignificantly active compared with the "specially prepared chlorophylls."

The composition of these chlorophyll extracts is not known. Apart from the fact that in the tests reported no descriptions of the preparations have been given, it is very difficult to analyse extracts of this kind. The water-soluble chlorophylls which have been marketed for many years as colouring agents contain the alkali metal salts of the acids (chlorophyllins) which result from alkaline hydrolysis of chlorophyll (chlorophyll is esterified by a methyl and a phytol group). Only the salts are soluble in water. The free acid and the ester are very insoluble. In the known extraction processes much of the magnesium of the original chlorophyll is likely to have been removed, and copper may have been substituted. Methods of standardization have been developed for measuring the colour only, but simple methods for analysis of the constituents are not available.⁹

If, as seems likely, the proprietary preparations recommended to combat odour contain chlorophyllin salts they would be precipitated in the stomach as the insoluble free acids. The extent of absorption and the metabolic fate of chlorophyll and its derivatives in man have not been fully elucidated. Certainly a large part is excreted as phaeophytin in the faeces. Brugsch and Sheard¹⁰ recovered 77% in this form in one case and 43% in another when 400 mg. of crystalline chlorophyll-a was given. The remainder could not be accounted for. Degradation products related to phylloerythrin were found in the bile, but it was not certain that they arose from the ingested chloro-

phyll. Urinary coproporphyrin was increased only from 80.6 μ g. to 113.6 μ g. by chlorophyll feeding, whereas that in the faeces increased from 48.6 μ g. to 770 μ g.

The action of chlorophyll in the tissues can only be surmised. Glasser¹¹ quotes a report¹² that chlorophyll increases respiratory metabolism in a way similar to thyroxine. Westcott⁸ showed that the water-soluble extracts removed the smells of certain chemicals—benzyl mercaptan, sodium thioglycollate, and trimethylamine—when incubated for five minutes at body temperature. Dijkstra¹³ found that the smells of rotting cheese and vitamin preparations were removed by incubating with soluble chlorophylls for 24 hours at 50° C. Chlorophyll derivatives might therefore modify metabolism or react with specific compounds, such as strong-smelling sulphur compounds, with a resulting decrease in odour. But this can be no more than a guess about the mode of action of a substance which is unidentified, is difficult to characterize and to trace in the body, and is claimed to produce an effect which cannot be assessed in any way but by subjective impression.

BASIS OF CHROMATOGRAPHY

One of the earliest achievements of chromatography was to show that the russet tints of autumn leaves are present even in spring, beneath the green mask of chlorophyll. All the colour of a young leaf will dissolve in an organic solvent such as petrol. To continue this experiment one then took a long glass tube and packed it nearly full with a dry powder such as aluminium oxide, set the tube vertically in a clamp, and poured the solution of leaf pigments into the top. Slowly and evenly the solution percolated into the powder, and as the last drop sank into the column clean petrol was poured on top and also began to percolate down. Eventually it began to drip out of the bottom of the tube below the packed oxide. If one then looked to see what had become of the leaf colour, one saw a series of coloured bands at different points along the column, all slowly moving to the bottom, but at different rates. Nearest the top and slowest were two green bands, the chlorophylls; in the middle came the reddish-brown xanthophyll, while fastest of all and nearest the bottom was the yellow carotene. Ultimately the different substances thus revealed in the green leaf emerged separately in solution from the bottom of the column. Chromatography is thus a technique by which coloured substances can be made to declare their individual presences in a mixture, and by which they can be separated and purified. Since this experiment of nearly 50 years ago other solvents have been tried and other powders used to make the column. But very few powders have been

¹ Balston, J. N., and Talbot, B. E., *Guide to Filterpaper and Cellulose Powder Chromatography*, 1952, London.

² Gross, J., and Pitt-Rivers, R., *Lancet*, 1952, 1, 439.

found to attract coloured substances as they move down the column, and still fewer are sufficiently selective in their attractions to make separations practicable. So adsorption chromatography—separation by differential attraction to the surface of a powdered solid—has not had a very wide use.

Just about 12 years ago Drs. R. L. M. Synge and A. J. P. Martin (see annotation on next page) invented a new form of chromatography. Instead of attraction to a solid surface they used attraction into water as a separation process. But water will not form a column like a dry powder: it is too cohesive to let another solvent penetrate it, and too restlessly fluid to avoid at once mixing together any separated substances. So to make a "water column" it was necessary to provide an inert scaffolding, to use damp shredded paper, or, better, silica gel, where the silica acted merely as a skeleton to hold the water still, erect, and penetrable. If mixed colours in an organic solvent were poured on the top of this column again a series of separated, slowly moving coloured bands appeared, but this time it was solubility in water which held the colours back. The more soluble they were, the more they were attracted out of the moving organic solvent into the stationary water. The extent to which a substance distributes itself between each of any pair of solvents is called its partition coefficient, and since even similar substances nearly always have different partition coefficients a separation is nearly always possible by this partition chromatography. The new method therefore has a much wider application than the old.

Even colourless substances can be made to show on the column provided they are acid or alkaline. It is only necessary to add a little indicator beforehand to the silica gel—for instance, a little bromcresol green, which gives it a bluish colour. Suppose now a solution of mixed fatty acids (acetic, propionic, butyric, and so on) in chloroform is put on the silica gel column. As it penetrates downwards the acids are extracted to different degrees into the stationary water layer, and being acids they mark their presence by turning the indicator yellow. Thus in a little while a series of yellow bands appears on the blue column, slowly moving down to the exit. They move because the organic solvent is moving and because each acid never stays finally in the water but all the time apportions its existence between the two solvents. When a little almost pure chloroform goes by some of the acid moves back into it, and all along the column, as the conditions alter, such exchanges are going on, back and forth, so that whatever quantity of acid is present it is always strictly apportioned between the two solvents in accordance with its partition coefficient.

This new chromatography has already proved immensely valuable in the isolation of vitamin B₁₂ and of antibiotics and is providing the key with which to lay bare the detailed structure of such proteins as A.C.T.H. and insulin. In a simple modification, paper chromatography, it is becoming of use to the clinical pathologist and clinician. In this a strip of blotting-paper or filter-paper replaces the silica jelly in its glass tube. A tiny drop of the solution to be studied, perhaps

an extract of urine or plasma, which has been freed of interfering organic salts, is placed at a marked place at one end of the paper strip and allowed to dry. Then that end of the paper is dipped into a trough of organic solvent such as butyl alcohol or collidine and the whole system enclosed in a box or jar to prevent evaporation and disturbance. Slowly the organic liquid creeps along the paper, and as it passes the dried spot it draws the mixed substances with it; they travel at different rates according to their partition coefficients, for there is enough moisture in the paper to provide a water phase alongside the organic solvent. After several hours the substances are spaced at different points along the paper, and if they are colourless they can sometimes be revealed by ultra-violet light or radioactivity, or by chemical test. The distance each one has moved, compared to the distance travelled by the solvent front in the same time, is a ratio called the R_f number, which is characteristic for the substance in that solvent. Thus an unknown substance may be identified by comparing its R_f numbers in several solvents with those of pure substances. This avoids the need also to present photographs of each experiment carried out.

Paper chromatography has already been used to study sugars, vitamins, fatty acids, nucleic acids, and other substances of medical interest in plasma, urine, plant juices, and tissue extracts.¹ In conjunction with radioactive iodine it has made possible an examination of all the iodine-containing compounds present in normal plasma, from which it appears very likely that the true thyroid hormone is not thyroxine but 3.5.3'-L-tri-iodo-thyronine.² It is a cheap, simple method which is very little bother and suitable for very small quantities. It can be only a matter of time before it is standard equipment in every large hospital laboratory.

WINTER SLEEP

Where do flies go in the winter-time? The answer is rather an anticlimax after the song. Those that do not die creep into cracks and crevices and sleep the winter out. So do the butterflies that make their appearance in the early spring. The snail seals up the door of its shell; the earthworm burrows deeper into the warm earth. Some fishes bury themselves in the bottom mud when their water freezes; others remain active until the water solidifies around them and survive the trap, provided they are not held too long and the temperature does not fall further. The danger to most living creatures is that the water of their tissues will crystallize as ice and destroy the structure of their cells. But its freezing-point may be lowered below that of fresh water by the presence of salts and other dissolved substances: proteins in particular have a restraining effect on ice formation and encourage supercooling. And so it is that even the Antarctic has life¹—mosses and lichens, mites, insects, and crustaceans—wherever the water melts for a short time.

It is the warm-blooded creatures like man which cannot stand the cold. Rabbits, cats, dogs, guinea-pigs, monkeys, and rats have been cooled to (rectal) tempera-

tures of anything from 19° C. (66° F.) to 10° C. (50° F.) and have died.² Patients with painful malignant disease have been cooled to about 28° C. (82° F.)—cryotherapy—at which temperature they are unconscious and the pulse and blood pressure imperceptible, so that it seems likely that much lower would kill them. At least one factor in the death of cats and rats is that their nerves cease altogether to conduct impulses at a temperature of 9° C. (48° F.). The animal therefore becomes disorganized when its body cools to this temperature.² The higher mammal is an animal needing a certain constancy of internal environment. It functions only with a steady body temperature, a steady blood glucose level, a steady osmotic pressure and ionic composition; perhaps higher cerebral function is possible only with these constant conditions, for life does not demand them. Primitive mammals, such as echidna, the spiny ant-eater from Australia, have only a rudimentary temperature control,³ and tend to cool off when the weather gets cold. Bats, strangely enough, have no control at all,⁴ and are really cold-blooded mammals. But in order to fly in cool weather they have to work up a body temperature by some minutes of shivering, and in real cold they sleep.

Going to sleep for the winter is a way of economizing on food. True hibernation is confined to certain rodents and insectivores, such as the dormouse, hedgehog, ground squirrel, marmot, and golden hamster. During the spring and summer they are normal warm-blooded mammals, but in the autumn their temperature control begins to break down and they sleep, at first for odd days, then more regularly, finally all the time. Body temperature now drops as environmental temperature drops, and the physiological responses are all changed. The respiratory centre becomes insensitive to carbon dioxide, which accumulates in the blood, for the animal may take only one breath in three minutes. The blood-sugar level drops, and the animal slowly metabolizes its body fat, in particular a special adipose tissue between the shoulder-blades. The heart beats very slowly; in the hamster with a normal beat of about 500 per minute the rate may fall to as low as 8.⁵ In the hamster, and probably in other hibernators, the nerves continue to conduct down to 2° C. (35.6° F.).

But this sleep is not absolute. Marmot and ground squirrel waken occasionally to defaecate, and sometimes to look for food. All will waken if they are in danger of freezing, while electric shocks, sudden heat, and even noise will also startle them into heat production. This is usually very rapid; the dormouse's body temperature has been known to rise 19° C. (34° F.) in just over 40 minutes. Part of the natural waking stimulus in spring seems to be finding food again. A dormouse normally sleeps about six months, but in a cold laboratory and without food it has continued over a year.

But environment is not the only factor in hibernation. The thyroid, the pituitary, and in particular the adrenal cortex show a cycle of histological changes throughout

the year. Hypophysectomy has resulted in hibernation, though followed in a few days by death,³ and it is possible that the hypothalamus also was damaged. It is thought that the temperature-regulating centres of the hypothalamus control hibernation⁵ through the pituitary and adrenal cortex, damage to which leads to hypothermia in man. But much research remains to be done on hibernators, and its results may well clarify our understanding of temperature control in patients.

NOBEL PRIZE FOR CHEMISTRY

The announcement last week by the Swedish Academy of Science that two British biochemists Dr. A. J. P. Martin and Dr. R. L. M. Synge are to share this year's Nobel prize for chemistry is an occasion for congratulation. The award, valued at £11,400, is for their work on partition chromatography, which was done in collaboration at the laboratories of the Wool Industries Research Association in Leeds. Martin and Synge now work separately, the former at the National Institute for Medical Research, Mill Hill, and the latter at the Rowett Research Institute, Aberdeen.

The importance of their invention was quickly recognized, and both were elected Fellows of the Royal Society in 1950. Their achievement consists essentially in the inspired application of a few simple physical principles to problems of separation. Even at its present stage of development chromatography has provided one of the most powerful analytical tools at the disposal of chemical science; by no methods other than those coming under its general heading can closely similar substances be separated from one another with such efficiency (see annotation on page 1087). In the hands of Martin and Synge themselves and in those of other workers the methods evolved have so far found their most effective application in biochemistry; it was indeed interest in the long-standing problem of separation of amino-acids that provided the initial stimulus for the work, and it is in this particular field that chromatography has achieved some of its greatest triumphs. The ramifications of all that is now understood by chromatography penetrate, however, into every corner of chemistry and even into atomic research. Since the effective chemical and biological study of natural products can be undertaken only after their complete purification, the importance of chromatography for the advance of chemical, biological, and medical research is self-evident. Many problems have already been solved by its aid; many more even more subtle will undoubtedly be solved, though an approach to their solution could not even have been contemplated had it not been for the work of the Nobel Laureates in Chemistry for 1952.

We announce with regret the death on November 8 of Air Vice-Marshal Sir David Munro, Director of R.A.F. Medical Services from 1921 to 1930, and Secretary of the Industrial Health Research Board from 1930 to 1942.

¹ Bertram, G. C. L., *Arctic and Antarctic*, 1939.

² Chatfield, P. O., *et al.*, *Amer. J. Physiol.*, 1948, **155**, 179.

³ Kayser, C., *Biol. Rev.*, 1950, **25**, 235.

⁴ Hock, R. J., *Fed. Proc.*, 1951, **10**, 65.

⁵ Chatfield, P. O., and Lyman, C. P., *Amer. J. Physiol.*, 1950, **163**, 566.