COLCHICINE WITH SPECIAL REFERENCE TO ITS MODE OF ACTION AND EFFECT ON BONE-MARROW.

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THE alkaloid colchicine was isolated in 1887 by Houdé and its action was investigated by Jacobi. Jacobi showed that it becomes changed in the body to oxydicolchicine and that whichever of these two bodies, colchicine or oxydicolchicine, is injected into an animal, symptoms of poisoning do not appear till from two to three hours after injection. He[1] showed further that colchicine excites some portion of the nervous mechanism of the stomach and intestines, causing vomiting and diarrhœa, and that it ultimately produces death by paralysing the respiratory centre.

Colchicum is a remedy of unquestioned value in the treatment of acute gout; it relieves the symptoms in such a remarkable way that its action has been termed specific. Formerly this beneficent effect was attributed to a greater elimination of uric acid, but more accurate knowledge has completely demonstrated that colchicum has no action on the excretion of uric acid. Nor has there been any valid hypothesis which accounts for its value in gout, unless it be that of Rossbach[2], who believes that the alkaloid paralyses the peripheral and central nerve terminals.

IMMEDIATE EFFECTS OF COLCHICINE.

Colchicine is remarkable among drugs in that it exerts two distinct types of action. It produces certain specific effects immediately, like most alkaloids; and it exerts certain remote effects only after it has been in the circulation some hours, like the toxins. The immediate effects are exerted on plain muscle and resemble those of pilocarpine. The

[1] *Arch. f. exp. Path. u. Pharm.* XXVII. p. 119.

[2] *Pflüger's Arch.* XII. p. 308. 1876.

automatic movements of plain muscle throughout the body are augmented: thus, peristalsis is increased, splenic, uterine, and bronchiolar muscle show augmented tonus and movements, and in every case the action is antagonised by atropine. In this respect it closely resembles pilocarpine and muscarine in its action, but it differs from these drugs in that it does not excite glands, nor the cardiac vagus. Even in the largest doses the heart is little altered, and blood-pressure is affected to a slight extent only. Fig. 1 shows the typical effect of injecting colchicine into the circulation of a mammal. The blood-pressure falls as the result of very slight cardiac inhibition, and the effect is not obtained on the atropinised animal. Fig. 2 shows the effect on the peristaltic movements of injecting colchicine; the most noticeable feature is the great rise in tonus which it causes, the action is almost identical with that of pilocarpine. Fig. 3 shows the effect of colchicine on the bronchioles. The amount of air entering and leaving the lungs was measured by the plethysmographic method, and the figure shows that about two minutes after the injection of the drug there is marked constriction of the bronchioles.

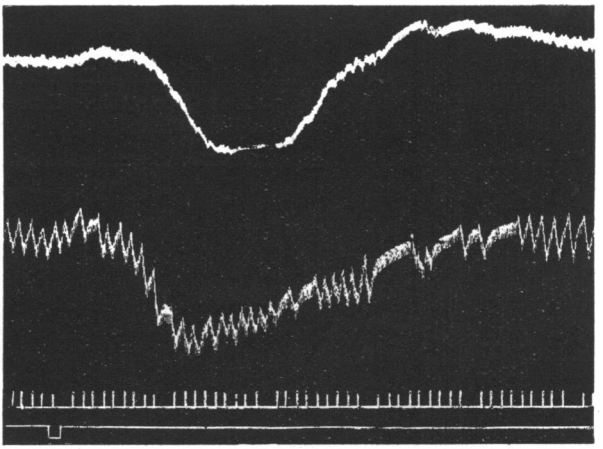


Fig. 1. Dog. Morphine. Urethane. Right limb volume. B.-P. The right sciatic nerve is severed. Shows the effect of injecting 1 c.c. 1 % colchicine into the left jugular vein. Time = secs.

There are several substances which are said to excite the nerve-endings to glands and plain muscle, principally because their effect is destroyed by the previous injection of atropine but not by nicotine; the cells are not affected by the atropine since they respond to direct stimula-

tion. Muscarine, pilocarpine, physostigmine, and colchicine are examples of such drugs. The proof that atropine paralyses "nerve-endings" is clear enough in the case of muscle. For example, it paralyses the fibres of the third nerve to the circular muscle of the iris: this muscle does not respond even when the nerve is excited post-ganglionically, yet the

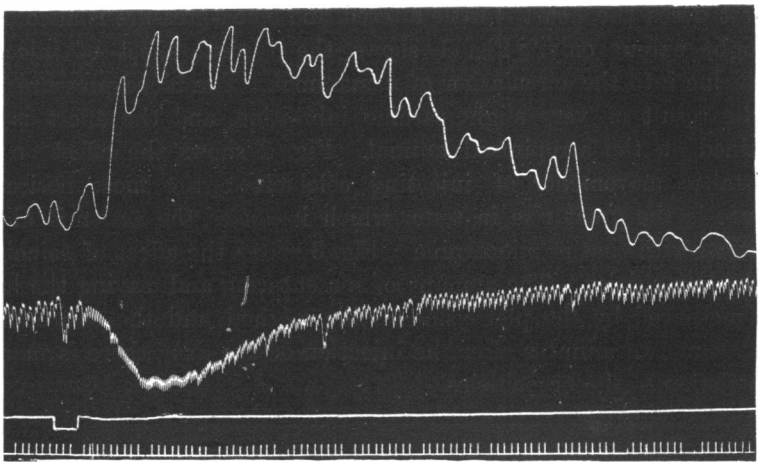


Fig. 2. Cat. A.C.E. Intestinal movements recorded by the balloon method. B.-P. Shows effect of injecting 1 c.c. $\frac{1}{2}$ % colchicine into a vein. Time = secs.

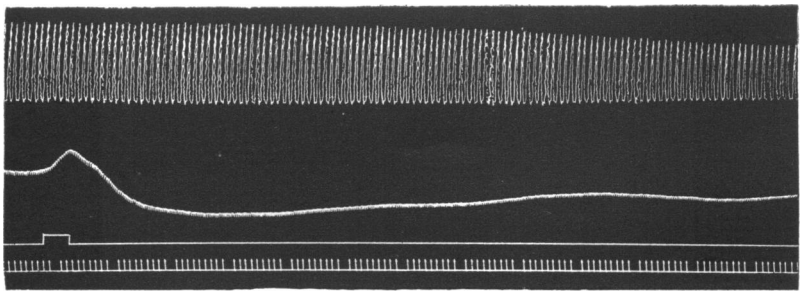


Fig. 3. Cat. A.C.E. Lung volume and B.-P. Artificial respiration. Shows effect of injecting 1 c.c. $\frac{1}{2}$ % colchicine. Time = secs.

muscle can be made to contract by the direct application of the electrodes to it. In the case of glands the proof is less convincing, since it does not appear to be possible to excite the gland cells directly by means of electricity. Atropine paralyses the chorda tympani to the submaxillary gland, so that no secretion of saliva is obtained even when the terminals

are pushed right down into the hilus of the gland (post-ganglionic excitation). Stimulation of the sympathetic nerve, unless atropine has been given in maximal doses, still causes some flow of saliva, so that the cells can still respond to a suitable stimulus.

There are reasons for believing that these drugs, physostigmine, pilocarpine and colchicine, do not act on the same structures, and also that the peripheral autonomic nervous system is not composed of chemically identical nervous elements. Colchicine, for example, exerts the typical effect on the "vagus endings" in the alimentary canal, but has little or no action on the iris, heart or vessels: nor does it excite except in the most trifling manner gland cells. Again, pilocarpine and physostigmine act differently on the plain muscle. This can be shown in the simplest manner on the enucleated eyes of frogs placed in normal saline solution in the dark. A 1 per cent solution of either pilocarpine or physostigmine constricts the iris till the pupil is pinhole. If the eyes are placed for about half an hour first in a 0·5 per cent. solution of atropine and subsequently in the pilocarpine and physostigmine solutions respectively the pilocarpine is now found to have no action and the pupil remains widely dilated, whilst the physostigmine constricts the pupil as before. It has been argued that physostigmine only differs from pilocarpine in that it has a much more vigorous stimulant action than the latter drug. But in view of recent work on muscle, which tends to show that degrees of activity depend upon the number of cells acting, this is a little difficult to understand but will receive further attention presently.

If frogs' eyes are placed for half an hour first in nicotine or curari, and subsequently in pilocarpine or physostigmine solutions, it is found that the pilocarpine acts as before, that is it constricts the pupil, whilst the physostigmine has almost no action; in other words pilocarpine can always act in the presence of nicotine and curari, but not in that of atropine. Physostigmine can act after atropine, but not after nicotine or curari.

Nicotine and curari paralyse the actions of all the nerve fibres which pass from the central nervous system to muscle, striped, plain, or cardiac, although the seat of action is more peripheral in the case of striped muscle. Atropine on the contrary paralyses some nerves only, such as the vagus to the heart and bronchioles, and the ciliary nerves: it has no apparent effect on the motor nerves or the splanchnic nerves as the result of electrical excitation, although it paralyses some structures or substances at the "nerve-ending," since the effects of pilocarpine and colchicine are not obtained if there has been a previous injection of

atropine. We mention these points here, not because we wish to discuss the question of "nerve-endings," but to support our general contention that an end organ may be excited peripherally in several ways, and that probably pilocarpine, physostigmine and colchicine all act on some different constituent and in a different manner.

If the dose of colchicine be very large, then the effect on plain muscle may be so severe as to cause collapse and death from this cause, but if the dose be not so excessive the animal is not unduly influenced by the alimentary disturbance and death does not ensue until some four or five hours later from gradual paralysis of the central nervous system.

Remote effects.

Besides this immediate action of colchicine there is a second action which comes on very slowly and is shown as a gradual paralysis of the central nervous system. In this respect, as in many others, colchicine will be found to bear a close relation to snake poison, and the toxins. An animal injected with this drug will remain perfectly well for three or four hours, and then gradually pass into a state of narcosis in which the reflexes disappear and which is followed by death from vaso-motor and respiratory failure. Fig. 4 represents such a condition. A rabbit of 1500 grms. was given a subcutaneous injection of 0·02 grm. of colchicine: four hours later it had become completely unconscious and the reflexes had disappeared. A blood-pressure curve was then taken from its carotid artery and a record of its respiration made by strapping a tambour to its chest. Fig. 4 *B* shows the condition five minutes later, the respiration is slower, and the blood-pressure has fallen. *C* shows a still later effect in which the respiration is exceedingly slow, and the inspiration very prolonged. In spite of the partial asphyxia the blood-pressure is falling. *D* shows the final respirations and death. At *E*, apparently both the heart and respiration had ceased, but adrenalin greatly raised the blood-pressure and so improved the circulation that the respiration returned for a time. This effect of colchicine is not due to a simple depression of the respiratory centre, for in that case it should be possible by doing artificial respiration, in such a stage as that represented in *E*, to raise the blood-pressure and generally improve the condition of the animal. But artificial respiration causes very little improvement in the circulation, and the primary cause of death must be looked for in the enormous engorgement of the abdominal vessels. It will

be noted in this experiment that death occurs about five hours after the first injection. If the colchicine be injected directly into the circulation of an anæsthetised animal death occurs generally in about 4 to 4½ hours after.

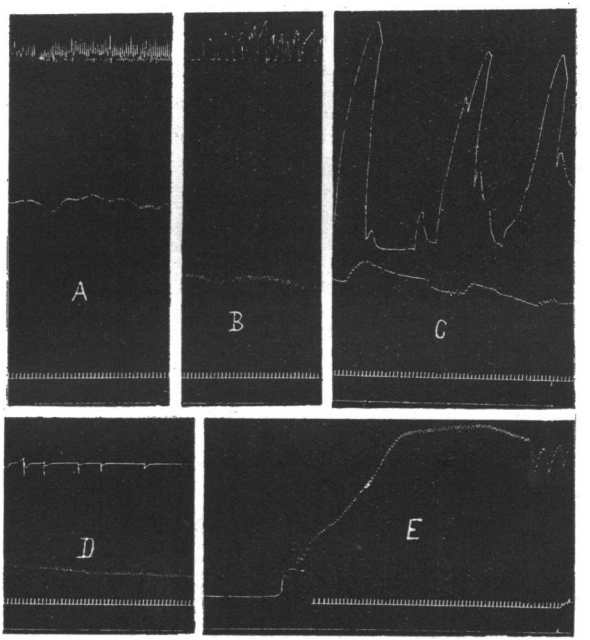


Fig. 4. Rabbit. Ether. Four hours previously had received 0·02 grm. of colchicine. *A* shows resp. and B.-P. 4 hrs. after injection, *B* 5 mins. later, *C* 25 mins. later, and *D* shows apparent death 4 hrs. and 40 mins. after injection. Artificial respiration produced no improvement but the injection of 1 c.c. 1 in 20,000 adrenalin caused the effect shown at *E*. Time = secs.

In Fig. 5 are represented the respiration and blood-pressure of an anæsthetised cat. At *A* the state of the blood-pressure and respiration is represented after the brain has been punctured a considerable number of times with a blunt needle through a small trephine hole made for the purpose. The colchicine was now injected into the circulation. *B* shows the effect four minutes later: respiration is slower but deeper, and blood-pressure is raised. *C* is another representation nineteen minutes after injection, the respiration is slow and shallow and the blood-pressure is considerably raised as the result of asphyxia.

At *D* fifty minutes after injection the blood-pressure is beginning to fall, and *E* sixty-three minutes after injection, respiration is obviously

failing, death being shown at *F* from vaso-motor paralysis one hour and twenty minutes after the first injection. This experiment then shows

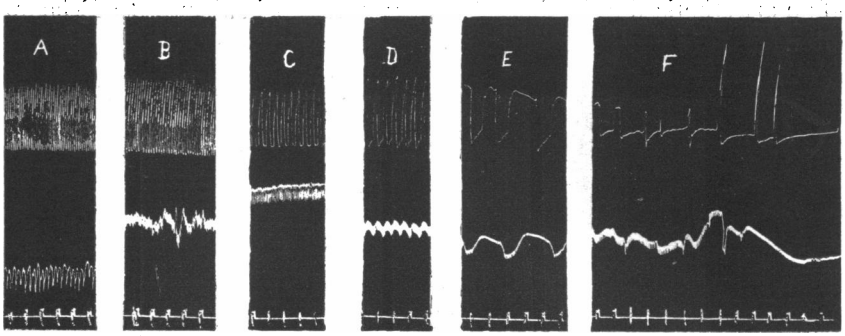


Fig. 5. Cat. A.C.E. The brain was punctured in a number of places just before 3 o'clock. *A* shows the condition of respiration and B.-P. at 3 p.m. soon after an intravenous injection of 0·03 grm. colchicine. *B* shows the effect at 3.4, *C* at 3.19, *D* at 3.50, *E* at 4.3 and *F* at 4.20 when death occurs.

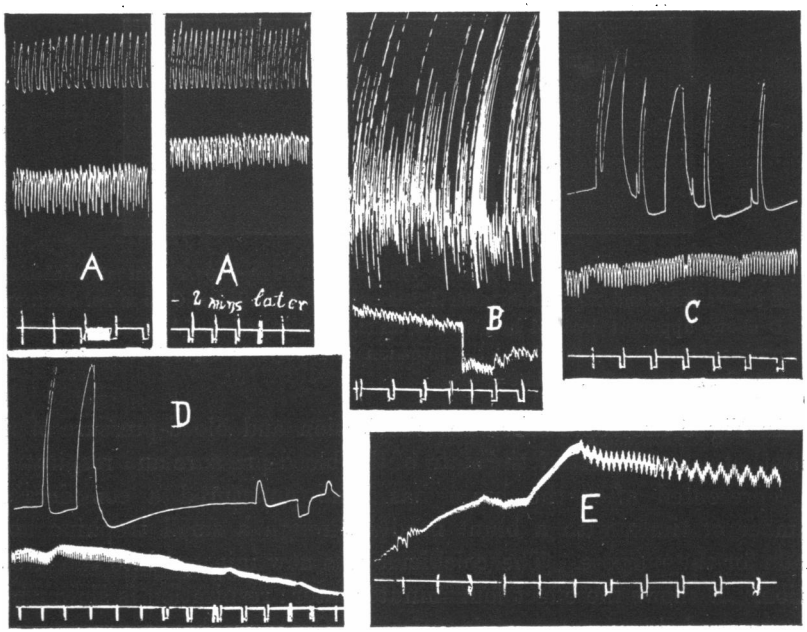


Fig. 6. Cat. C.E. 2 c.c. of ½ % colchicine was injected through a trephine hole directly into the cerebral hemispheres at *A*. *B* shows the effect 1½ mins. later; *C* shows the effect 35 mins. after injection, and *D* shows death from respiratory failure and splanchnic vaso-dilatation 1 hr. after injection. At the beginning of tracing *E*, 1 c.c. 1 in 10,000 adrenalin was injected into the left jugular vein.

that if the brain be punctured in various positions, colchicine is able to act much more rapidly than under normal conditions. It should be noted that puncturing the brain in this manner in the case of anæsthetised animals does not cause the effect which we ascribe to the colchicine.

A further type of experiment is exemplified in Fig. 6. In these experiments the cat was anæsthetised with chloroform and ether and a small trephine hole made as before; through this hole 2 c.c. $\frac{1}{2}$ % colchicine was injected directly into the brain substance (cerebral hemispheres). The immediate effect of such an experiment (as shown in Fig. 6) is to raise blood-pressure and to increase the rapidity and depth of respiration. $1\frac{1}{2}$ minutes later (shown in *B*) the respiration has become very irregular and rapid, and blood-pressure has fallen. Thirty-five minutes after injection vaso-motor paralysis is evident, respiration is obviously failing, and the blood-pressure is very low, this is seen at *C*; *D* shows the death of the animal from vaso-motor and respiratory failure one hour after injection. The respiration is completely paralysed, and cannot be recovered, although the vaso-motor system still responds to an injection of adrenalin as at *E*; the circulation never recovers by artificial respiration alone.

Colchicine in its action on the central nervous system would seem then to present some analogy with the toxins. The neurones are protected from the poison, at all events for a time, by the walls of the blood vessels and lymph spaces, and if it is desired to allow the drug to act quickly either it must be injected directly into the brain or it must be injected in the ordinary way and lesions made in the cerebral substance by means of some body such as a needle. In the case of tetanus toxin we know that it is the motor nerve fibres which provide the mode of access of the toxin to the central nervous system, and the incubation period is occupied by the carriage of the toxin along the motor nerves to the centre. We have not been able to prove that colchicine is absorbed into the nerve centres in a similar way to tetanus toxin; our experiments are not conclusive, but we nevertheless regard it as not unlikely that some such process of absorption occurs in the case of colchicine.

THE ACTION OF COLCHICINE ON THE BLOOD.

Colchicine we have found exerts a profound effect on the blood and bone-marrow, and this effect is therefore dealt with in some detail. Our experiments have been made on rabbits, rats, dogs, and in a few cases

upon men, and the drug has been administered usually by subcutaneous injection.

Method. In the rabbit the blood was collected from the marginal vein of the ear, and in the dog also in the ear from one of the small veins. Leucocyte counts were made with the Thoma-Zeiss instrument, and blood films were prepared on cover slips, and stained usually by Jenner's method. Preparations of bone-marrow were made from the freshly killed animal, either in the case of sections from the ends of the femur or in the case of smear preparations from the femur or ribs. Bone-marrow for cutting was hardened either in alcohol or perchloride of mercury, embedded in paraffin, and stained with hæmatoxylin and eosin.

Experiment I shows a typical effect caused by injecting colchicine into a normal rabbit.

Exp. I. Rabbit. Wt. = 2100 grms. Injected at 10 a.m. subcutaneously with 1 c.c. 0·5 % colchicine.

Time	Number of leucocytes per c.mm.	
10 a.m.	5400	
10.50	3370	
11.35	1870	
12.5 p.m.	2810	
1.0	4700	
2.30	7500	
4.40	18700	
5.20	23100	animal killed and tissues hardened.

In this experiment a rapid fall in the number of the leucocytes follows the injection. This hypoleucocytosis obtains from one to three hours according to the amount of drug administered, and is always followed as in this experiment by an increase in the leucocytes till a maximum hyperleucocytosis is established in from 10 to 24 hours after injection. After about 36 hours the leucocytes gradually diminish in numbers and reach their normal in about 48 hours. In appropriate doses the animal suffers no discomfort, and remains perfectly well during the whole experiment. Smaller doses gave similar but less marked results.

The next experiments were undertaken to determine the manner in which the different varieties of leucocytes react to colchicine, and the numerical relationship which they bear to each other. This is shown typically in the following experiments (II and III), also on the rabbit.

But before giving the details of these experiments the differential count of the blood of normal rabbits must be noted; the following

numbers are the results obtained from an analysis of the blood of 21 normal rabbits four or five hours after food.

Lymphocytes	50—55 %
Large mononuclears...	3—4
Polymorphonuclears...	40—50
Eosinophils	less than 1
Basophils	3—5

Exp. II. Rabbit. Wt. = 2050 grms. Injected subcutaneously with 0·01 grm. colchicine.

	Normal blood	1 hr. after injection	2¼ hrs. after	24 hrs. after
Total leucocytes	8125	3750	2031	16300
Polymorphonuclear and eosinophil cells	58 %	9 %	10 %	79 %
Lymphocytes and mast cells ...	42 %	91 %	90 %	21 %

Exp. III. Rabbit. Wt. = 2250 grms. Injected subcutaneously with 0·01 grm. colchicine.

	Normal blood	3 hrs. after injection	21 hrs. after
Total leucocytes	9844	7200	19375
Polymorphonuclear and eosinophil cells	54 %	17 %	82 %
Lymphocytes and mast cells	46 %	83 %	18 %

It is evident from a number of such experiments that the stage of hypoleucocytosis is caused almost entirely by a diminution in the number of the polymorphonuclear leucocytes, the decrease in the lymphocytes being relatively insignificant. Three hours after injection, in most experiments, the leucocytes in the peripheral circulation had nearly reached the normal numbers, and this increase was due chiefly to the lymphocytes, although there was some small recovery in the polymorphonuclear variety. The lymphocytes decrease a little during the stage of leucopænia and later increase, but throughout they are less affected than the other leucocytes. Four hours after injection the total leucocytes in the circulation are greater than the normal, and eight or ten hours after the number has increased generally to 18,000 or 20,000 per c.mm., chiefly owing to lymphocytes which may now represent 75 % of the total white cells. This lymphocytosis gradually disappears and yet the total number of leucocytes in the circulation keeps up and even increases. Eighteen to twenty-four hours after injection the leucocytosis reaches its maximum, and this is now found to be due almost entirely to an increase in the polymorphonuclear variety. Colchicine leucocytosis in the rabbit is, at first, then due to lymphocytosis and later, and for a much longer period, to the polymorphonuclear variety (see Fig. 7).

After such large doses of colchicine as these (0·01 grm.), abnormal

cells are present in the blood. Normoblasts are invariably found; one or two are usually found in the "hypo" stage, but their numbers gradually increase: thus in Exp. II, 2¼ hours after the injection, twelve normoblasts were observed in a count of three hundred leucocytes. Neutrophil myelocytes are present in the "hyper" stages. In Exp. III they were first seen three hours after injection and their

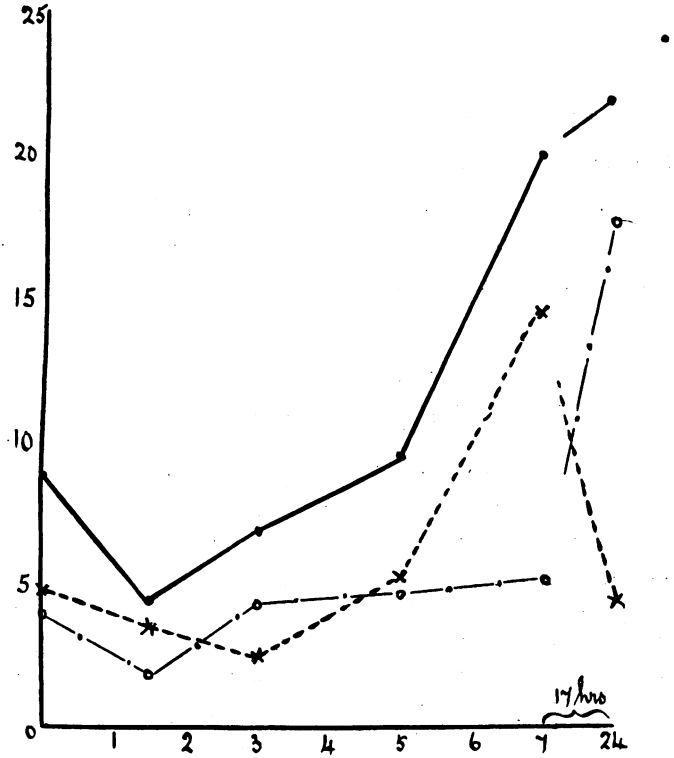


Fig. 7. Diagrammatic representation of differential leucocyte count in the rabbit (see text). Ordinate = number of leucocytes in thousands. Abscissa = time. The black line represents the total number of leucocytes per c.mm. in the peripheral circulation: the dotted line represents lymphocytes, and the interrupted line granulated cells.

numbers gradually increased with the hyperleucocytosis. In the hypo and early hyper stages many of the polymorphonuclear corpuscles were found ruptured: their nuclei appeared to be swollen, did not take the aniline dyes properly, and their granules were extruded. This effect was constantly noted in all experiments in which large doses of colchicine were given. This condition of the leucocytes may be due either to the

poisonous action of the drug on the cells, or to a large increase of immature cells turned out from the bone-marrow, and which are less able to withstand pressure than the normal leucocytes. We do not think that the former view can be entertained, because isolated polymorphonuclear leucocytes in Ringer's solution are not affected in this way by the addition of colchicine in amounts that might be present in an animal's blood after it had received a subcutaneous injection of a drug. The cells under discussion bear a very close resemblance to the immature leucocytes of the bone-marrow; this is seen especially in the character of the ruptured cells, which both in appearance and staining properties resemble those of an immature cell. Moreover, we do not regard the rupture of cells and extrusion of the granules as being caused by faulty preparation of the specimens, for exactly similar effects may be obtained in the preparation of bone-marrow smears, and furthermore it sometimes happens that free granules are found in the blood. On the whole then the evidence points to these leucocytes being immature cells from the bone-marrow and not the normal cell of the blood altered by the toxic action of the colchicine.

Exp. IV. Rabbit. Wt.=1800 grms. Injected subcutaneously with 0·02 grm. colchicine in normal saline solution at 1.5 p.m.

	1 p.m.	1.30	3.0	5.0	9.15
Total leucocytes per c.mm. ...	8850	4600	6700	9650	20,000
Eosinophil %	1	1	1	0·5	1
Polymorphonuclear ...	37	16	50	36	16
Large mononuclears ...	10	4	7	4·5	3·8
Lymphocytes	45	75	32	45	71
Mast...	7	4	7	6·5	1
Myelocytes	—	—	3	7·5	7
Normoblasts	—	5	4	8	40
Megaloblasts	—	—	—	—	1
			*	†	‡

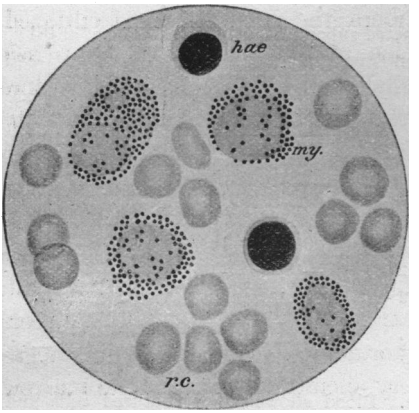
* A few ruptured polymorphonuclears. † Ruptured leucocytes much increased.
‡ Nearly all polymorphonuclears are ruptured.

Exp. IV (Fig. 7) shows a more complete differential count taken at various periods after a very large dose of colchicine. It is remarkable in this experiment that four hours after injection the myelocytes formed 7·5 % of the total leucocytes in the general circulation, whilst 8¼ hours after injection, in a count of 300 leucocytes, forty normoblasts and one megaloblast were seen. Moreover eight hours after injection very few normal polymorphonuclear leucocytes could be found, they were nearly all of the immature bone-marrow type and ruptured. The

very large dose of colchicine administered in this experiment has greatly delayed the onset of polymorphonuclear leucocytosis, and the large increase of cells at this time (8 hours after) is entirely due to lymphocytes, although 24 hours after injection (see Fig. 7) the leucocytosis is of the polymorphonuclear variety. There can be no doubt from this and other experiments that colchicine in these large doses is a powerful poison to certain forms of leucocytes. It tends, at first, to force all leucocytes from the peripheral circulation, but soon the more hardy lymphocytes are attracted back in increasing numbers, the process being facilitated, no doubt, by the contracted condition of the spleen and lymphatic glands and at a time when the circulation is still unfit for their more delicate fellows the polymorphonuclear variety. As the action of the colchicine still further passes off and relaxation of plain muscle, including that of the spleen and lymphatic glands, occurs, the attraction for the lymphocytes ceases, and they leave the free circulation. It is at this time, when the lymphocytes are passing out from the circulation, that the attraction for the polymorphonuclear cells becomes so evident. They collect in the general circulation in ever increasing numbers and are associated with myelocytes, normoblasts, and occasional megaloblasts. Indeed at this stage every element of the bone-marrow may be represented in the general circulation. Many of these cells, which we have classified as polymorphonuclears, and in which the nuclei were not fully divided, were clearly in a transitional stage from myelocytes: several of them were larger than normal and oval in shape, and the granules were collected at one pole and not uniformly distributed as in the adult leucocyte.

The blood picture at the point of maximum hyperleucocytosis is strongly suggestive of some powerful influence exerted by the drug on the bone-marrow whereby its elements are swept out into the general circulation. This action may be one either directly on the bone-marrow of a positive chemiotactic nature or of some disturbance of the regulating mechanism of the hæmatopoetic organs which normally prevent the passage into the blood stream of immature elements. Whichever of these views be correct the fact remains that at this period the blood of the general circulation contains every variety of cell in every stage of development, which is usually present only in the bone-marrow (Figs. 8 and 9). This action of colchicine is not confined to the leucoblastic cells, but also affects the erythroblastic as megaloblasts, normoblasts, free nuclei and megalocytes are all found and may be present in considerable numbers. Much polychromasia is always

found in these cases: the significance of this is not understood, but it is generally regarded as being due to degeneration of the cytoplasm. It may be caused either by the toxic effect of the drug acting directly on the corpuscles or to the fact that imperfectly developed and therefore rapidly degenerating elements are hurried into the circulation. Polychromasia usually persists long after all other signs of abnormality have disappeared from the circulating blood.



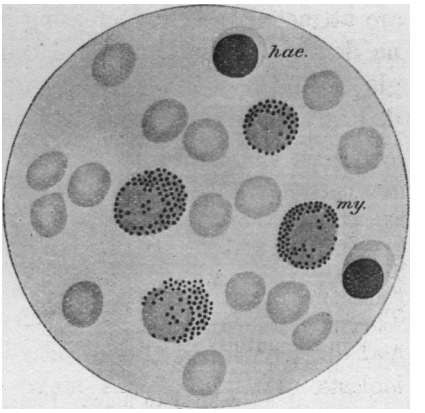


Fig. 8. Represents a typical blood smear taken from the rabbit used in Exp. V, nine hours after injection. This field shows four myelocytes (*my*) and two nucleated red cells (*hæ*). Jenner's stain.

Fig. 9. Represents a bone-marrow smear from the femur of the same rabbit (see Exp. V) on the following day. Similar cells are seen to those in Fig. 8. (Staining in every detail was as in Fig. 8.) The types of all the cells seen in this preparation have their counterpart in Fig. 8.

As rabbits are particularly susceptible, as regards changes in their leucocytes, to almost all foreign substances, similar experiments were repeated on other animals, namely on the rat, the dog, and on man. In all these experiments injections of colchicine exert the same type of action on the blood. Rabbits are remarkably tolerant to colchicine relatively to the body weight as compared with other animals. The comparatively large dose which must be given to rabbits to induce leucocytosis is, no doubt, as will be shown later, responsible for the differences which obtain between them and other animals.

In Exp. V the effects on the leucocytes are of the same character as those observed in the rabbit, but are spread over a longer period. The dog and all carnivorous animals, including man, are much more susceptible to colchicine than herbivorous, such as the rabbit. Hence in the case

of the dog it is necessary to inject only one-tenth the dose per kilogramme body weight as compared with the rabbit. One reason for this is no doubt due to the depressant effect of colchicine on the higher cerebral centres. So far as our experiments go they suggest that the toxicity in different animals varies according to the degree of development of the cerebral hemispheres. That is to say, the dose of colchicine for any animal may be gauged by determining the relationship between the body weight and weight of its cerebrum.

Exp. V. Dog. Weight 3650 grms. was injected subcutaneously with 1·25 c.c. of 0·1 % of colchicine dissolved in normal saline solution (Fig. 10).

	Normal blood	4½ hrs. after injection	10 hrs. after	21 hrs. after	30 hrs. after	54 hrs. after	72 hrs. after
Total leucocytes	12,920	6,600	15,480	24,200	27,920	19,680	10,800
Eosinophil	8 %	5 %	4 %	1 %	1 %	1 %	1 %
Polymorphonuclear	45	48	62	79	78	68	56
Large mononuclear	17	16	15	4	6	16	25
Lymphocytes	30	25	15	9	13	14	18
Myelocytes	—	2	2	—	—	—	—
Transitional cells	—	4	2	7	2	1	—
No. of nucleated red cells in a count of 300 leucocytes	—	9 *	24 †	12	2 ‡	—	— §

* Animal disinclined to move. Diarrhœa. Blood shows a few ruptured cells and some polychromatic reds.

† Some remarkably large myelocytes observed. One megaloblast. Diarrhœa better, but still declines food.

‡ Slight diarrhœa. Taking food well.

§ Animal quite well. No abnormal cells.

The stage of hypoleucocytosis is prolonged for nearly four hours in the dog, whilst the stage of hyperleucocytosis does not reach its maximum until 48 hours after the injection, and the blood does not return to normal for yet another 48 hours. A further pronounced difference between the dog and rabbit is the fact that in the former the polymorphonuclear leucocytes are the variety which shows the marked changes throughout. The lymphocytes exhibit relatively insignificant variations in number, so that the total leucocyte curve is almost coincident with that of the polymorphonuclear corpuscles. This difference is apparent rather than real. In the rabbit the number of lymphocytes, at first, increases considerably, so that after eight or nine hours the increase of white blood corpuscles is largely due to lymphocytosis. But after 18 or 24 hours these corpuscles have returned to their normal numbers, whilst the polymorphonuclears have enormously increased in numbers, and it is this stage which represents the stage of hyperleuco-

cytosis in the dog. The difference between the two animals is due entirely to the early lymphocytosis which occurs in the rabbit and not in the dog. The cause of this difference may be simply explained. We have already pointed out that colchicine has two actions; the one immediate, due to stimulation of nerve-endings, and this is the same in all mammals, and varies with the dose: the other developing slowly,

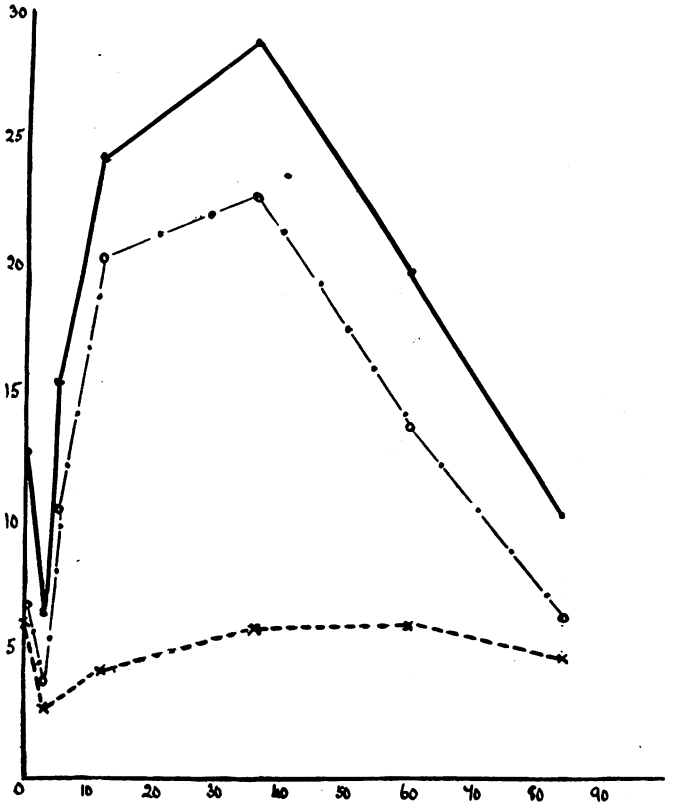


Fig. 10. Diagrammatic representation of leucocytes in the dog (see Exp. V). Ordinate = number of leucocytes per c.mm. in thousands. Abscissa = time. The curves are as in Fig. 7.

and animals vary in susceptibility to this action according to the degree of development of the cerebrum. To produce the same degree of poisonous action in the dog as in the rabbit it is necessary to inject only

about one-tenth of the dose: and as this dose would have comparatively little effect on nerve-endings, so there would be relatively little tendency to contraction of the spleen and lymphatic glands, and the immediate production of lymphocytosis. Moreover, if the rabbit be injected with much smaller doses of colchicine than those we have described, lymphocytosis is relatively less and polymorpho-leucocytosis relatively more marked. Still more important is the fact that the lymphocytes are a much hardier type of cell than the polymorphonuclear and they may be attracted into the general circulation when colchicine is present in an amount which would be quite prohibitive to the presence of the polymorphonuclear variety.

In the dog as in the rabbit abnormal cells appear during the hypoleucocytosis stage and continue to be present for two or three days. Normoblasts are invariably present, but not in such relatively large numbers as in the rabbit. The abnormal leucocytes consist of neutrophil myelocytes, and transitional cells, the latter at the end of 48 hours representing 7 per cent. of all leucocytes.

Several of the cells appear to have swollen nuclei, which take the aniline dye badly and some of the cells are ruptured; these changes are however not so prevalent as in the rabbit. Many of the red corpuscles show a considerable amount of polychromasia. Injections of colchicine into men cause a similar type of leucocytosis to that found in the dog.

The effect of atropine in controlling lymphocytosis.

It has been shown that any cause which brings about contraction of the plain muscle in the spleen and lymphatic glands produces an almost immediate lymphocytosis[1]. The injection of atropine into an animal by depressing or paralysing some constituent of the "nerve-ending" going to this muscle for a time eliminates the lymphocytosis. Thus pilocarpine induces no lymphocytosis after atropine, though the nerve is not necessarily paralysed to electrical excitation. In the case of the rabbit which is very tolerant to atropine, largely on account of the fact that it excretes the alkaloid so rapidly, the influence of the drug is only temporary.

[1] Harvey, *Journ. Physiol.* xxxv. 115. 1906.

Exp. VI. Rabbit. Weight 1900 grms. Injected subcutaneously with 1 c.c. of a 0·5 % solution atropine sulphate. This injection followed immediately by an injection of 1·5 c.c. of a 0·5 % solution of colchicine.

	Normal	½ hour after injection	1 hour after	3½ hours after	6 hours after	10 hours after	24 hours after
Total leucocytes	8,800	1,040	3,320	7,920	14,720	22,640	13,720
Eosinophil	1·5 %	0·5 %	1 %	0 %	0 %	1 %	2 %
Polymorphonuclear	40·5	28	22	38	32	34	40
Large mononuclear	7·5	3·5	3	4	2	2	1·5
Lymphocytes	47·5	62	71	54	63	57	37
Mast	3	6	3	8	1	1·5	·5
Myelocytes	—	—	—	1	2	4·5	20
Nucleated reds observed when counting 300 leucocytes	—	—	—	— *	4 †	14 ‡	8 §

* Many ruptured polymorphonuclear cells.

† Considerable polychromasia.

‡ Many horse-shoe nuclei in amphophil cells. These cells are swollen and in many cases have the granules extended.

§ Many ruptured polymorphonuclear cells. Much polychromasia.

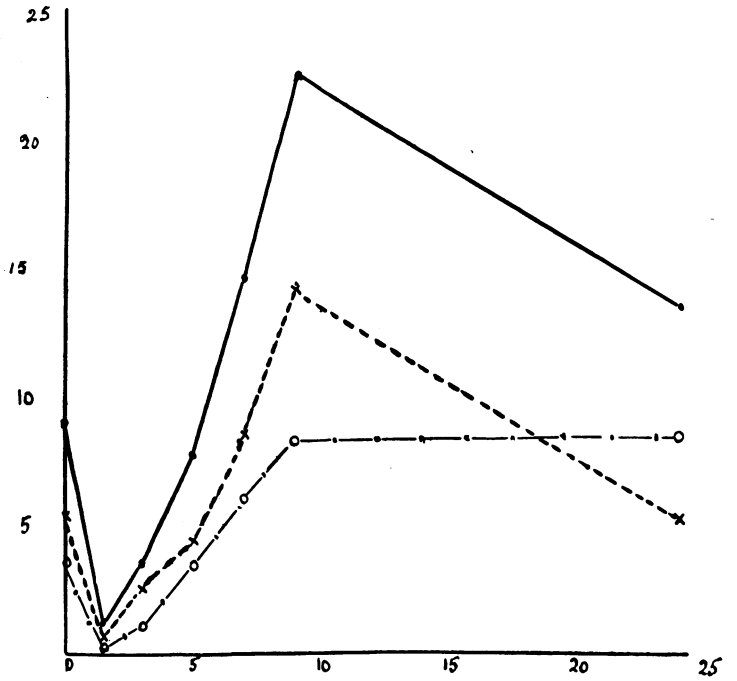


Fig. 11. Differential count of leucocytes in the rabbit after an injection of colchicine and atropine (see Exp. VI). Curves as in Fig. 7.

The results of this experiment closely resemble those in Exps. I and II. The stage of hypoleucocytosis reaches the minimum about one hour after injection, when all leucocytes show a marked diminution in numbers, but especially the polymorphonuclear variety. Very considerable hyperleucocytosis is reached nine hours after injection, and, as usual at this time in the rabbit, the lymphocytes are increased in greater proportion than the other varieties. Twenty-four hours after injection the leucocytes had fallen from 23,000 to 13,000 entirely as a result of diminution in lymphocytes, as the polymorphonuclear variety had increased. The abnormal cells found in this experiment are similar to those seen in Exps. I and II. The period of polymorphonuclear leucocytosis is not recorded in this experiment, but it was present from the 12th to the 24th hour after injection.

This experiment might seem at first sight to show that this lymphocytosis was not due to mechanical squeezing of the spleen and lymphatic glands. But this argument is untenable because of the rapidity with which the action of atropine passes off in the case of the rabbit. Whilst admitting, then, that colchicine excites the spleen, and so tends to get rid of its cells by mechanical squeezing, and that early lymphocytosis may be due to this cause, yet the late lymphocytosis which occurs about six hours after injection, can hardly be so caused. Colchicine exerts its action on plain muscle immediately and it is only late that the marked lymphocytosis occurs. So that this late lymphocytosis can be regarded as a change similar in nature to that which affects the polymorphonuclear cells. It has been pointed out already that the lymphocytes are hardier towards poisons than the polymorphonuclear cells. Thus it is possible that one percentage of drug may act positively to lymphocytes and negatively to other blood cells. If to this fact it is remembered that the dose of colchicine for a rabbit is ten times that for a dog, we obtain an explanation of the considerable lymphocytosis during the first eight hours after injection in the former animal and of polymorpho-leucocytosis in the latter.

THE EFFECT OF SMALL REPEATED DOSES OF COLCHICINE.

Further experiments were conducted to determine the effect of small doses of colchicine when repeated over several weeks; the results of these are seen in Exps. VII and VIII in the case of the rabbit, but the main features have been repeated on the rat.

The animals in Exps. VII and VIII showed no ill effects as the result of the colchicine, and they gained weight during the period of experimentation almost at the same rate as the control animals. It will be noticed that on May 4th the blood in Exp. VIII contained 6·5 % of myelocytes and transitionals, and many immature and often ruptured polymorphonuclear cells (see Fig. 12). The red cells showed in many cases basophil granulations, and polychromasia: whilst counting three hundred leucocytes six normoblasts were observed. On the twenty-first day this animal was killed and preparations of bone-marrow were taken from the ribs and femur.

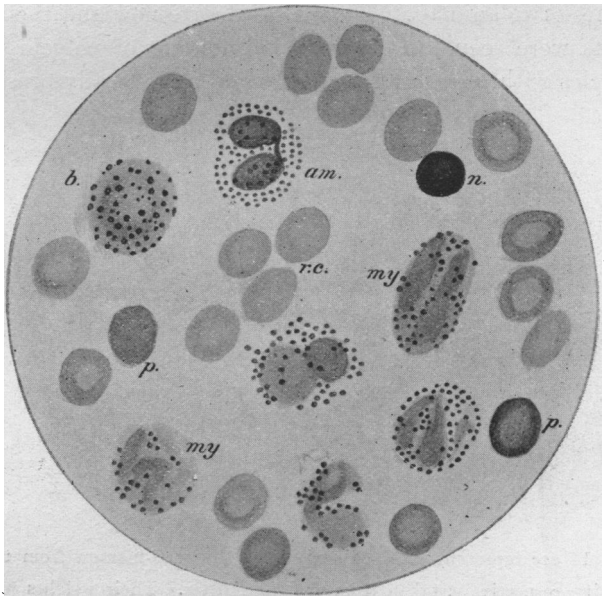
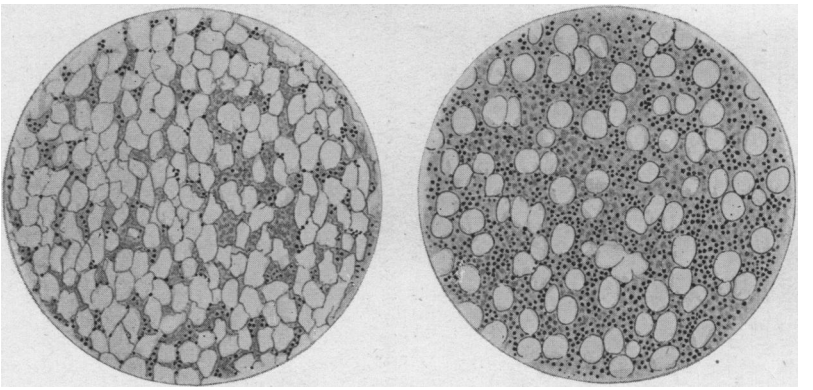


Fig. 12. Represents a blood smear from the rabbit used in Exp. VIII. It was taken on the fourth of May and shows the abnormal polymorphonuclear cells which were present in large numbers. These cells are transitional from myelocytes to the normal polymorphonuclear variety (*my*). One basophil cell (*b*) only is present in this field though these cells were subsequently greatly increased in numbers. *n* is the nucleus of a red cell and *p* cells showing polychromasia. *rc* = red corpuscles, *am* = amphophil cell.

During the whole period of Exp. VII the animal appeared in perfect health, and the leucocytes remained steady, the maximum number in

the circulation never exceeding 14,000 and the minimum never showing below 7500 per c.mm. The ratio of polymorphonuclear cells to lymphocytes also remained fairly constant. The most noticeable feature in the differential count from these two experiments is the large increase in the number of mast cells: they increased from 2 % in the normal blood to a maximum of 35 %, and at no period during the experiment did they fall below 14 %. This effect is a constant feature although we are ignorant of its significance. If the rat be treated with small doses of colchicine the large basophil cells normally found only in the peritoneal cavity become more or less plentiful in the blood.

Abnormal cells were occasionally, though not commonly, found in the blood in these two animals. Thus, a myelocyte now and then and a few normoblasts were seen. A considerable amount of polychromasia and red corpuscles with basophil granulations were generally present.



Figs. 13 and 14 are representations of sections of the bone-marrow from the femurs of two rabbits, respectively, taken from the same litter. Their weights were 1500 and 1250 grms. Fig. 13 was made 12 hours after an injection of 5 m. grms. colchicine and Fig. 14 one hour after a similar injection. All details as to hardening, cutting and staining, were identical in the two cases. (Eosin and methylene blue.)

BONE-MARROW CHANGES.

Sections have been made on the bone-marrow of rabbits under various conditions of leucocytosis. Figs. 13 and 14 show sections of the bone-marrow taken from the femurs of two rabbits from the same

Exp. VII. Rabbit, 9 weeks old, weight=760 grms. Injected with 0·0005 grm. colchicine on April 15th, 18th, 22nd, and with 0·001 grm. on April 26th, 29th, May 4th, 6th, 10th, 13th, 25th, 31st, June 25th, 27th, July 2nd, 5th, and on July 11th and 14th with 0·02 grm. On April 24th, the animal weighed 800 grms., on May 4th, 803 grms., on May 25th, 1000 grms., on June 25th, 1130 grms, on July 5th, 1350 grms., and on July 11th, 1430 grms.

Date...	Before treatment	Ap. 22	May 10	May 12	May 13	May 15	May 16	May 18	May 25	May 26	May 31	June 25	June 27	July 2	July 5	July 11	July 14	July 15
Total leucocytes	10,000	10,840	10,560	11,000	11,540	10,500	—	12,400	12,000	13,500	12,400	14,200	10,200	11,400	11,300	7,500	10,800	—
Eosinophil	4 %	1 %	— %	1 %	3 %	4 %	2 %	6 %	2 %	2 %	1 %	2 %	2·5 %	1 %	1 %	2 %	1 %	1 %
Polymorphonuclear	31	53	39	22	30	35	38	41	21	42	40	43	27	36	24	25	44	44
Large mononuclear	8	9	6	5	4	6	10	6	4	6	3	1	2·5	3	5	5	2	10
Lymphocytes	55	23	30	39	45	20	18	19	45	28	38	36	42	37	48	52	31	26
Mast	2	13	25	34	18	35	32	28	21	22	28	18	26	33	22	14	22	18
Myelocytes	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			*	†	‡	§	‖	¶	**	††	‡‡	§§	‖‖	¶¶	***	†††	‡‡‡	

* One nucleated red cell seen.

† Much bursting of polymorphonuclear and mast cells. Much polychromasia and many basophil red cells.

‡ Ditto.

§ Ditto. One myelocyte seen.

‖ No myelocytes or nucleated reds but many basophil reds and much polychromasia. A few ruptured polymorphonuclear cells.

¶ Two nucleated red cells. Not so much polychromasia.

** No abnormal cells.

†† One nucleated red cell seen. No polychromasia.

‡‡ No abnormal cells.

§§ No abnormal cells.

‖‖ No abnormal cells.

¶¶ A few ruptured polymorphonuclear cells. Nothing else abnormal.

*** No abnormal cells.

††† One nucleated red cell seen.

‡‡‡ A few polychromatic reds but no other abnormal cells seen.

litter. The sections were prepared, cut, and treated in the same way in every detail, except that in Fig. 14 the animal was killed one hour after an injection of 0·5 c.c. of 1 % colchicine at a time when the leucocytes had diminished in the circulation from 10,000 per c.mm. to 1800: whilst in the other (Fig. 13) the sections were made twelve hours after a similar injection of colchicine when the leucocytes had increased from 10,000 per c.mm. to 19,000 per c.mm. In the latter case the marrow appears to be largely denuded of marrow cells, whilst in the former the section is teeming with them. Sections obtained from normal animals also from the same litter showed a condition as regards the number of cells intermediate between these two but bearing a much closer resemblance to Fig. 14 than to Fig. 13.

Exp. VIII. Rabbit, 9 weeks old. Weight 730 grms. Injected subcutaneously with 0·0005 grm. colchicine on April 15th, 18th, 20th, 22nd, and with 0·001 grm. colchicine on April 24th, 26th, 29th, May 4th, and May 6th. The weight on April 24th, was 808 grms. and on May 4th, 840 grms. Leucocyte counts were made immediately before the injections.

Date...	Normal blood before treatment	April 29	May 4	May 9
Total leucocytes	10,300	11,200	—	8,080
Eosinophil	1 %	0·5 %	0 %	1 %
Polymorphonuclear	35	60	71	31
Large mononuclear	8	10·5	2	10
Lymphocytes	53	22	16	46
Mast	3	6	4·5	22
Myelocytes	0	1	6·5	0
		*	†	‡

* Some polychromasia swelling and occasional bursting of the polymorphonuclear cells of an immature type. Occasional nucleated red cells seen.

† Polychromasia marked. Some basophil reds, 6 nucleated reds seen in a count of 300 leucocytes, many abnormal transitional cells.

‡ No abnormal cells observed.

The disappearance of leucocytes in the stage of leucopænia following on the injection of toxins is generally attributed to their migration into the pulmonary, and possibly liver capillaries, although Löwit and others have suggested that their disappearance may be due partly to destruction. The lungs undoubtedly harbour some of these leucocytes after injections of colchicine; we have satisfied ourselves as to the increased

numbers of leucocytes in the pulmonary capillaries in the case of animals killed during the stage of leucopænia. We believe however that some of these leucocytes are driven back into the bone-marrow and the sections which we have reproduced in Figs. 13 and 14 are evidence of this. In view of the reputed toxic action of colchicine on undifferentiated protoplasm we at first thought it possible that the leucopænia might be the result of a mechanical sifting out of swollen and cohesive leucocytes by the capillary endothelium; but this is not the case since we have shown already that these altered leucocytes are in all probability immature cells from the bone-marrow. Moreover, as we have already pointed out, colchicine has no such direct action on the leucocytes. So that we are forced to the conclusion that the leucocytes are sent out of the circulation by some chemical influence. In the later stages evidence is conclusive that colchicine is a powerful stimulant to the bone-marrow, since it turns out into the circulation all the elements including the erythroblasts, and leaves the marrow relatively denuded of corpuscles.

After treating an animal with colchicine over a prolonged period certain definite changes are evident in the bone-marrow. The marrow cells increase in numbers and become more conspicuous, whilst the fat cells tend to disappear. The increase in the marrow cells is noticeable in the case of the finely granular oxyphil cells, and in the basophil cells, but especially in the latter. These cells show plentiful mitotic figures and appear to be undergoing multiplication. Not only then does colchicine in large doses empty the marrow, but in small doses it acts as a marrow stimulant and encourages the development of marrow cells.

Very large doses of colchicine undoubtedly produce destruction of all the elements of the blood, and we have already drawn attention to this fact as regards the polymorphonuclear cells. But the red blood corpuscles are also to some extent destroyed and blood serum obtained from an animal after a large dose of colchicine will be found to be tinged pink as the result of hæmolysis and the liberation of free hæmoglobin.

Effect on blood coagulation, temperature, etc.

Two other effects of colchicine require mention. The first of these is the time taken for the blood clotting. Coleman[1] at our suggestion

[1] *Journ. Biochem.* II. p. 184. 1907.

has investigated this point, and finds in the rabbit after a toxic dose of colchicine that the time taken for coagulation under constant conditions increases synchronously with the leucocytosis, and twelve hours after injection may be prolonged to four or five times the normal period. The curve of temperature also follows that of leucocytosis. The initial leucopænia is associated with a drop in the temperature, but as the leucocytes increase the temperature rises, ultimately reaching a point two to three degrees Cent. above the normal (Fig. 15).

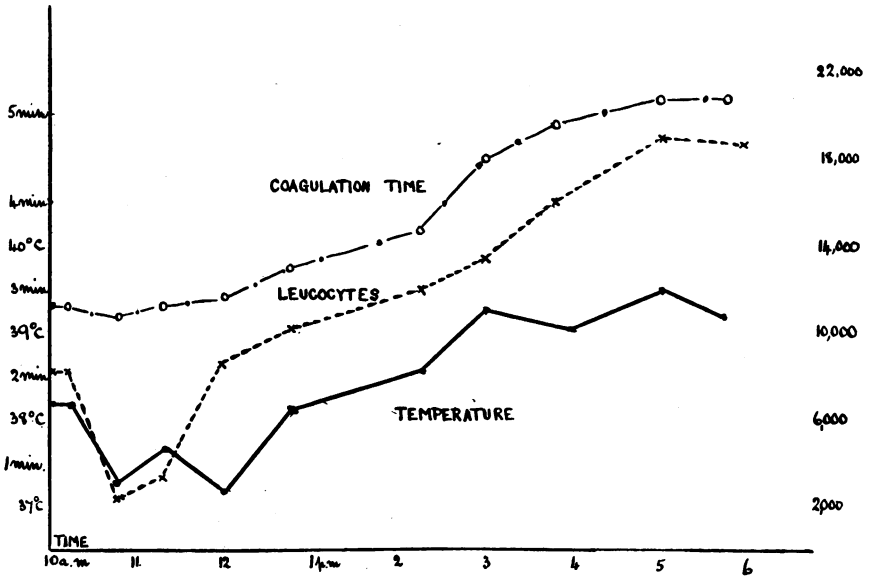


Fig. 15. Rabbit, 1870 grms. Injected subcutaneously 0·002 grm. colchicine. Shows the effect on blood-coagulation, leucocytes and temperature. Ordinates represent time of clotting of the blood, number of leucocytes per c.mm. of blood and rectal temperature respectively. Abscissa = time.

From the general description of the action of colchicine it will readily be seen how close are the resemblances in its action to those of the toxins. Its effect upon the central nervous system is very similar to that of cobra poisoning; its delay in absorption presents analogies with that of tetanus toxin, and its general effect on the blood shows a close similarity to the effect obtained by the injection of cobra and other toxins. If it be possible, then, for drugs to produce anti-body formation this would seem to be the drug. Attempts were made there-

fore to detect such anti-bodies in the serum of animals which had been previously dosed with the drug. For this purpose the precipitin test was employed: it was tried in the case of three rabbits but the result in each was negative. From the knowledge which we have recently accumulated concerning tolerance of drugs this is what would be expected. For it has been shown in this Laboratory that in the case of nicotine and morphine, tolerance is but the capacity of the tissues for destruction of the poison, and the animal whose tissues can most readily destroy the alkaloid is the most tolerant.

Conclusions.

1. Colchicine excites "nerve-endings" to plain muscle, like muscarine, but has little or no action on nerve-endings in the heart or in glands.

2. Pilocarpine, physostigmine and colchicine act on different constituents of the "nerve-ending."

3. Colchicine even in enormous doses is a slow poison and requires from three to six hours to cause death in carnivora: increasing the dose does not appreciably alter this time. Colchicine acts more slowly in herbivora than carnivora.

4. Delay in poisoning is due to slow absorption into the central nervous tissues (cf. tetanus toxin).

5. Death is caused by vaso-motor paralysis.

6. Injections of colchicine cause after transient leucopænia great leucocytosis in the rabbit, rat, dog, and man. In carnivorous animals this is due to increase in the number of polymorphonuclear cells; in herbivorous animals there is first lymphocytosis, succeeded later by leucocytosis of the granular cells.

7. After a large dose of colchicine all the normal elements of the bone-marrow are found in the general circulation. A large number of immature polymorphonuclear cells can be observed. Plentiful myelocytes and erythroblasts may also be seen.

8. During the stage of maximum leucocytosis sections of the bone-marrow show a distinct diminution in marrow cells as compared with sections from normal animals; conversely, during the stage of leucopæni marrow cells seem to be increased in numbers.

9. Repeated small injections of colchicine cause considerable increase in the basophil cells without directly affecting the other leucocytes. Sections or smears of the bone-marrow from animals so treated exhibit proliferation of the marrow cells: plentiful mitotic forms can occasionally be observed.

10. After injections of colchicine, plotted curves representing number of leucocytes, temperature, and time of coagulation, exhibit a general parallelism. Tolerance to colchicine is not caused by anti-body formation.

The expenses of this research were defrayed by a grant from the Royal Society.