Experimental Growth in Animals

9.1: Endocrinological Research

One of the most striking features of colchicine, whether injected into animals or acting upon tissue cultures,[23] is the accumulation of mitoses arrested at metaphase (Fig. 9.1). This is a consequence of the absence of spindle (cf. Chapter 3). The increase in the number of mitotic cells was soon understood to be most useful for the analysis of growth by cellular multiplication. Several lines of research were started in the years 1934–36. At this time, the isolation and the synthesis of hormones were proceeding rapidly, in particular, the steroid hormones of the sexual glands. These substances have most powerful physiological effects, the principal being to stimulate cells to increase the rate of appearance of new mitoses. Now, ordinary histological techniques give only an instantaneous picture of the state of the tissues at one given moment. If the cell divisions proceed very rapidly, there will be small chance of observing them in a microscopic slide. Colchicine, by arresting all these rapid cellular changes, would be able to let the mitoses progressively accumulate in a given tissue. Counting would be easier, and easier also the localization of regions of maximal growth.

While several authors understood the usefulness of colchicine as a tool for the study of growth, the largest amount of work was done in the field of endocrinology. Allen, Smith, and Gardner[2] are to be credited with the publication, in 1937, of an excellent paper with splendid photomicrographs that gave added impetus to research with this new technique. They were studying the action of estrogens in the mouse. After injecting the still chemically impure hormone of that type at their disposal, "theelin," they observed that colchicine increased tremendously the visible mitotic action in tissue sections. In the vaginal epithelium, they mention "a most incredible number of mitoses." * In a single transverse section of the vagina, controls in-

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* E. Allen, M. Smith, and W. V. Gardner, "Accentuation of the Growth Effect of Theelin on Genital Tissues of the Ovariectomized Mouse by Arrest of Mitosis With Colchicine," *Amer. Jour. Anat.*, 61 (1937), p. 324.

jected with "theelin" alone showed 20 to 30 dividing cells. After colchicine, this was increased to more than 1500 in about 10 hours. In one experiment in which "theelin" and colchicine were injected simultaneously, the authors wrote that "the general impression is that approximately every other cell is in mitosis."* These results aroused great interest, and marked one of the starting points for
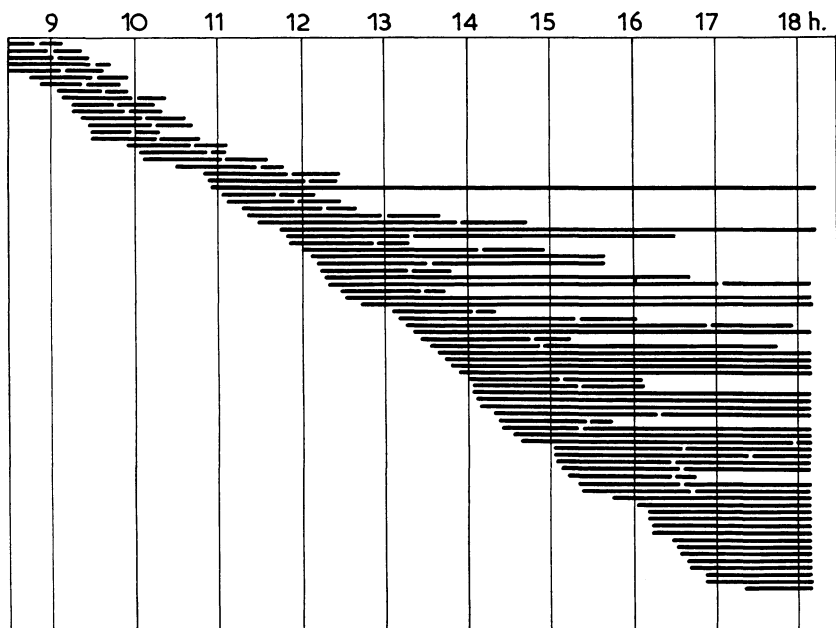

Fig. 9.1—Graphical representation of the course of cell division in a fibroblast culture treated by colchicine (1/20,000,000). During the two first hours, no notable changes. Later, progressive accumulation of arrested mitoses. Each horizontal line represents one mitosis; it is interrupted at the end of metaphase. Any vertical line indicates the number of visible mitoses at one moment, that is to say, the mitoses which should be seen in a fixed preparation. This number progressively increases under the influence of colchicine. The rate of apparition of new prophases is not disturbed with this concentration. There is no mitotic stimulation. (From a cine-micrographic recording. After Bucher, 1939)

colchicine research outside of the Brussels laboratory. Together with the discovery of colchicine polyploidy in 1937, this study initiated the publication of a great number of papers in which colchicine was mainly considered as a *tool* for making mitotic growth more visible and easier to analyze.

However, any tool has its advantages and its shortcomings. Many workers do not appear to have considered carefully the fundamental problems involved in what Allen called the "freezing" of mitoses.

* *Ibid.*, p. 325.

Some of the complexities have already been scrutinized in the first chapters of this book. A few more considerations about this particular problem of multiplying the numbers of mitoses by destroying their spindle will be useful for future workers in this field. While the number of papers published about the colchicine method appears to be on the decrease, so far as can be assessed, for colchicine is not always mentioned in the titles, much work remains to be done. This chapter will point out several unexplored fields.

9.2: Theoretical Considerations

Most of the American authors, following the first papers of Allen, those of Brues[19, 20, 21, 22] on liver regeneration, and the tissue culture work of Bucher[23] and Ludford,[62] considered colchicine simply as a means of stopping any mitosis at metaphase. The complexities of colchicine pharmacology (Chapter 7) should alone call for more caution.

A. P. Dustin, Sr., in a paper published in 1936, but which could not have received much publicity, demonstrated the utility of colchicine as a tool.[41] He had noticed the increased number of divisions in the wall of a parasitic cyst in a mouse, a fact which was the starting point for experiments on the healing of wounds, reviewed further on in this chapter. In his own words, "colchicine enables the detection of the otherwise invisible state of preparedness to mitosis." * It throws into an abortive division all the cells which are ready to divide, or had been prepared for mitosis, for instance, under the influence of endocrine or other stimuli. This was in agreement with the line of thought which had led to the discovery of colchicine's action in 1934, and which was the study of the regulation of mitotic growth.

The theories of "mitotic arrest" or "arrest after mitotic stimulation" are conflicting. In work where the location of mitoses is the main purpose and where no quantitative data are required, colchicine is useful whatever the opinion one has about a possible stimulation of mitosis. This problem, however, should not be overlooked. For instance, several authors have thought it possible to calculate from the number of mitoses found after colchicine, the average duration of these mitoses, had they not been arrested. This duration is, of course, an indication of the rapidity of cellular growth in the tissues studied. It should be clearly realized that such calculations imply several unknown factors, and they have a precise signification only if the following conditions are fulfilled:
1. Colchicine arrests all mitoses, shortly after it has been injected and until the end of the experimental period.

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* A. P. Dustin, "La Colchicine, Réactif de l'Imminence Caryocinétique," *Arch. Portugaises Sci. Biol.*, 5 (1936), p. 41.

2. The intermitotic period is much longer than the duration of the experiment, and is not modified by the experiment.
3. The arrested mitoses are not destroyed before the moment the tissues are fixed and examined.
4. The tissue is homogeneous from the point of view of mitosis, that is to say, mitotic rates and intermitotic periods do not vary from one region of the tissue to another.
5. The mitotic rate does not vary during the experimental period, in control animals.

Such conditions are not often fulfilled. One type of experiment in which they are is liver regeneration; this will be considered further. In mammals, cellular destruction is a factor which cannot be ignored. If, however, the above-mentioned causes of error do not exist, the average duration of mitosis can be found by the formula $A = Mt/X$, in which M is the mitotic index before colchicine, and X the index found t hours after the injection of the alkaloid.

If this formula is applied to the results obtained in the experiments referred to in the previous paragraph,[2] it is found that after "theelin" stimulation, the average duration of mitoses would be 10 minutes. This is a remarkably short period, and it may be questioned whether mitoses can be completed so rapidly. However, results obtained by A. P. Dustin, Sr., in the uterus of the rabbit after stimulation by chorionic gonadotropic hormones, are rather similar.[41] The increase in the number of mitoses was observed in repeated biopsies. Figure 9.2 shows that it was considerable, and that in one animal, the calculated duration of each mitosis, had it not been arrested by colchicine, would be 12 minutes. These results bring some evidence for mitotic stimulation, for the prophase mitotic index increased also. This indicates that more cells were undergoing prophase than expected; that is to say, a true stimulation took place. This index rose from 7.56 to 14.8 in 2 hours, and from 4.8 to 24.4 in 7 hours. It must, of course, be supposed here that the duration of each prophase was not affected by colchicine.

Such results are rather complex, for the mitotic index could have been modified by the traumatisms of the biopsies themselves, and also by the continued action of the hormone. The possibility of a synergic action of hormones and colchicine cannot be ruled out[66] (cf. Chapter 7).

The following results[29] are all the more interesting, for while they apparently could demonstrate such a synergism, a much simpler explanation is possible. Table 9.1 gives the results of mitotic counts in the seminal vesicles, after stimulation by a single large dose of testosterone. There appears to be a veritable "explosion" of mitoses, to use the expression coined by A. P. Dustin, Sr. Does this give evidence of mitotic stimulation by the alkaloid? The counts of the con-

trol animals demonstrate that it does not, for it can be seen that between the thirtieth and thirty-fifth hours after the hormone injection the mitotic index rises sharply. If colchicine had been injected at the thirty-first hour, a mitotic increase from 2.92 to 108.60 would have been observed, and this could not be explained by the theory of metaphase arrest. This increase is, however, not only the result of mitotic
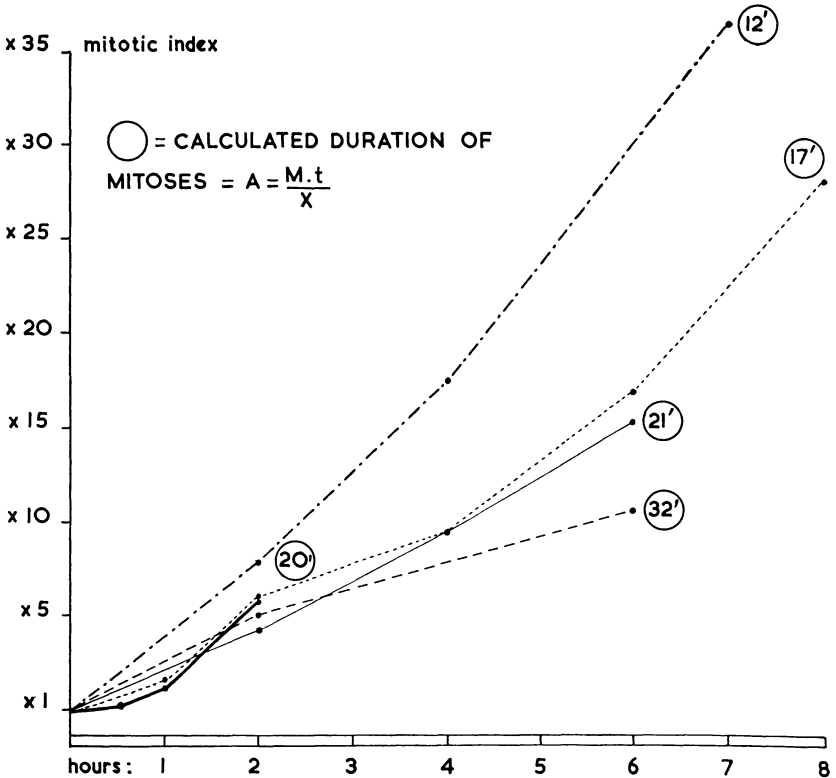

Fig. 9.2—Progressive increase of the numbers of mitoses, in repeated biopsies from the rabbit's uterus, after stimulation by chorionic gonadotropins and injection of colchicine. Calculated duration of mitoses on the assumption that colchicine does nothing more than arrest them at metaphase. (From original data of A. P. Dustin, 1943[41])

stasis, but also of the progressive action of testosterone, demonstrated by the fact that in untreated animals the mitotic count rises about threefold. Therefore, colchicine alone has increased the mitoses only from about 10 (2.92 × 3) to 108.60 within 4 hours, which means that the average mitotic duration must be about 25 minutes or less. This agrees with knowledge of mitotic duration in mammals.

Such an example demonstrates the intricacies of quantitative

work with colchicine. Others will be found in this chapter. Here, as in other fields of colchicine work, problems must not be over-simplified, and here especially, the greatest care should be taken in all quantitative estimations. It is striking that it is when colchicine is considered as a tool that the need for fundamental knowledge is the most apparent.

9.3: Cellular Multiplication in Normal Growth

Growth patterns in the organs of adult animals can be revealed far better after colchicine than with ordinary tissue sections. The alkaloid may do more than simply locate the germinative zones of organs; under strict experimental conditions, it may solve some quantitative problems of growth. Another method, which has brought excellent results, is to study the growth of explanted tissues. This has been done by the ordinary methods of tissue culture,[23, 62, 88] or

TABLE 9.1

MITOTIC ACTIVITY IN THE SEMINAL VESICLES OF CAS-
TRATED 80-DAY-OLD RATS TREATED WITH 0.3 MG. OF
TESTOSTERONE PROPIONATE
(Abridged from Burkhart[29])

Time After Treatment (*hours*)	Control	Colchicine
15...........	0	0.04
19...........	0	0.24
23...........	0.28	0.20
27...........	5.00	2.04
31...........	*2.92*	7.60
35...........	10.68	*108.60*

by a modified technique in which cellular multiplication was ob-served only for a few hours after explantation.[5-8, 24-27] Some of the results demonstrating how useful colchicine may be as a tool in such work will be summarized here.

9.3–1: Studies in vivo. Some of the early work in this field was done on the ovary. Colchicine, by increasing from 11 to 35 times the number of mitoses that could be observed in the germinal epi-thelium of the ovary of mice, demonstrated that this was a region of active growth.[1, 38, 14, 80] Similar facts were observed in guinea pigs.[76, 77] The relation between the mitotic activity in the ovarian follicles

and the estrus cycle were carefully analyzed (Fig. 9.3). In the endothelial cells of the theca interna of the ovarian follicles, immediately before ovulation, the karyokineses were found to increase about sixty-fold. Arrested mitoses of follicular cells in the rat can be found around eggs after they have reached the uterus (Fig. 9.4).[4] Some follicles are found to be growing rapidly while others are quiescent.
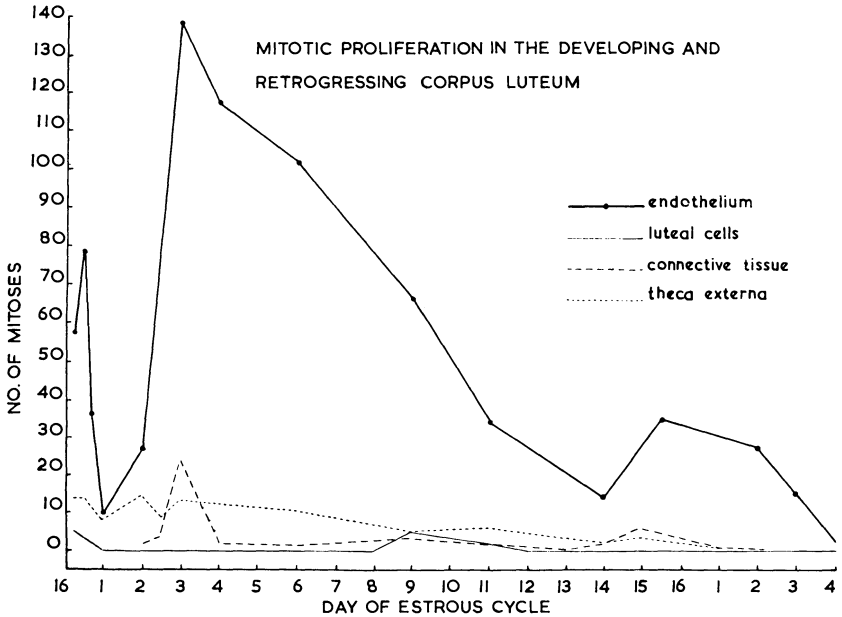

MITOTIC PROLIFERATION IN THE DEVELOPING AND
RETROGRESSING CORPUS LUTEUM

endothelium
luteal cells
connective tissue
theca externa

NO. OF MITOSES

DAY OF ESTROUS CYCLE

Fig. 9.3—Mitoses in the corpus luteum of the ovary of a normal mature guinea pig, studied by the colchicine method. (After Schmidt[77])

This fact is not evident in control animals, because the number of mitoses is too small.

In the pituitary glands of mice, colchicine increases the number of mitoses about threefold. This is an indication that these mitoses are normally of long duration. Many data have been gathered about the mitotic activity in this organ in various physiological conditions. [9, 52] Table 9.2 shows how evident is the action of age on mitotic activity when the number of metaphases has been artificially increased by spindle poisoning.[52]

A quantitative study of cell regeneration in the mucosa of the intestine in rats has been made possible by colchicine. It was known that the intestinal cells are continuously shed, but how long it took for the whole epithelial lining to be replaced was not known. Table

9.3 gives the results, with the percentages of dividing cells and of mitotic stages in control and colchicinized animals.[60] From these results, it is apparent that mitotic arrest at metaphase has increased in six hours the number of cell divisions by 17.63/3.32. The mitotic duration, calculated as indicated in Section 9.2, is $3.32 \times 6.0/17.63 = 1.13 = 1$ hr. 8 min. It can be calculated from this result that in 37.7 hours (1.57 days), 100 per cent of the cells will have divided; that is to say, a complete renewal of the epithelium will have taken place. This is, of course, only statistically correct, for there must remain a certain number of stem cells so that growth may persist. These cells will divide into one differentiating cell and one stem cell identical to the first. A great discrepancy between results obtained with radio-phosphorus on the nucleic acid turnover and the figures given by the colchicine method as used by the same authors has been discovered.[81] This may throw more light on the complex problems of growth in differentiating tissues.

The skin of small rodents has been excellent testing material for the study of growth as analyzed by colchicine. A very extensive series
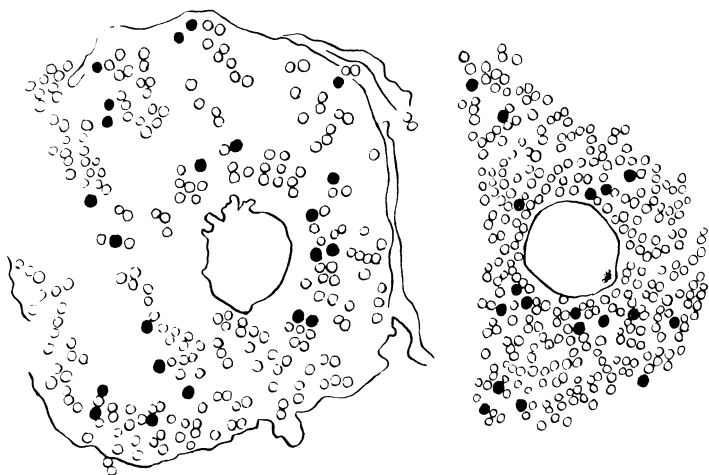

Fig. 9.4—Colchicine-mitoses (black dots) in an ovarian follicle (**left**), and in follicular cells surrounding an egg found in the uterus in the rat. (After Allen et al.[4])

of experiments has been carried on, especially by Bullough.[24-27] This has provided ample material for a precise analysis of growth and the fundamental mechanisms of mitosis. Further reference will be made to some of these papers in the section on hormonal stimulation of mitosis. Diurnal variations, the action of sleep, the effects of blood-sugar level and of injections of starch, have led to the most im-

portant conclusion that carbohydrate metabolism is indispensable for mitosis in epidermal cells, and that it provides the energy necessary for a cell to initiate division. Once prophase has started, no further energy requirements are apparent, and mitosis proceeds as if it were an all-or-none reaction.[24, 26] These experiments have also shown that the mitotic increase after colchicine corresponds to a

TABLE 9.2

EFFECT OF AGE ON MITOTIC ACTIVITY IN THE PITUITARY GLANDS OF FEMALE RATS
(After Hunt[52])

Age (days)	Pituitary Mitoses (per sq. mm.)
96	77.5
148	45
188	32
220	15
300	5

normal duration of about three hours. This is very long compared to that of ten minutes mentioned in Section 9.1. The difference may be partly explained by the action of hormonal stimulation, which not only increases the number of new cells starting to divide but also apparently shortens the duration of mitosis. This will be considered in a subsequent paragraph. Some other complexities of the study of epidermal growth and of the action of colchicine can be understood by the fact that the alkaloid may decrease the number of new mitoses,[24] and that unless observations are made within six hours after the injection of the alkaloid, some arrested metaphases may proceed to telophase.

TABLE 9.3

DIVIDING CELLS (PER CENT) IN THE ILEAL EPITHELIUM OF MALE RATS
(After Leblond and Stevens[60])

	Per Cent Nuclei Undergoing Mitosis (Normal and Abnormal)	Stages (per cent)				
		Prophase	Metaphase (Normal)	Metaphase (Degenerating)	Anaphase	Telophase
Controls	3.32 ± 0.35	24	36	0	5	35
Colchicine	17.63 ± 0.82	2	57	41	0	0

These studies of the epithelial growth in mice lead to a most interesting development which will now be considered: the study of growth in explanted tissues.

9.3–2: Growth in vitro. Many of the fundamental discoveries related to colchicine-mitosis were made on tissue cultures.[6, 23, 61, 62, 54, 88, 90] The importance of metaphase arrest in increasing the number of visible mitoses without modifying the mitotic rate has been illustrated by Figure 9.1. Other results on the action of colchicine on neoplastic cells in tissue culture, and on the mitosis-arresting properties of colchicine derivatives and other mitotic poisons will be related in Chapters 10 and 17. Tissue culture work offers definite potentialities for further investigation. The utilization of synthetic or semisynthetic media and the roller-tube technique are some of the modern aspects of tissue culture which could benefit from colchicine.

On the other hand, most important results have been obtained by simplified methods in which surviving tissues are utilized. Within the short duration of the experiments, mitoses proceed normally, and problems of bacterial contamination, transplantation, and dedifferentiation do not arise. These methods have been used in the study of the skin and bone marrow of mammals, including man.

As a consequence of previously mentioned work on the skin of the ears of mice, Bullough[24] developed a technique of *in vitro* study of the mitotic activity. *In vivo* experiments had demonstrated that glucose[26] and oxygen[27] were indispensable for providing the energy required for cell division. Glutamate was further demonstrated to increase the rate of cell division. The *in vitro* method should eventually bring forth important new data on the metabolic requirement of epidermal cells. Colchicine increases the amount of visible mitoses and makes counts simpler. However, because of the long duration of cell division in this type of tissue, colchicine does not produce any of the spectacular increases which have been seen in other organs. An important result was to establish that a linear relation existed between the number of arrested mitoses and the oxygen tension. While only 0.4 mitoses could be seen in pure nitrogen, the figures were 3.9 for 60 per cent nitrogen and 40 per cent oxygen, and 8.3 in pure oxygen.[27] The general significance of these results is made clear by nearly identical findings with bone marrow cells.[5] This work has been done mainly in Italy. Astaldi and a group of collaborators first studied the colchicine response of human bone marrow.[6] This is readily available by sternal puncture, and colchicine has provided a new insight on the growth of this tissue. This growth is far more rapid than that of skin; in mammals, bone marrow and intestinal mucosa are the tissues which have the highest mitotic index. After explantation, small fragments were kept at 37°C. in human serum,

and their growth could be studied for as long as 36 hours. The number of mitoses was considerably increased by colchicine, and the authors have indicated that this "stathmokinetic index," as it has been called, may throw considerable light on many problems of normal and neoplastic cellular division. Some of these will be mentioned in Chapter 10.

Very small amounts of colchicine are effective; dilutions of 1:1,000,000 were used. The alkaloid may disturb slightly the normal maturation of cells of the erythroblastic series. This is only visible after 12 hours *in vitro,* and for most experiments, important data can be recorded from 4 to 8 hours after colchicine. The action of embryonic extracts[7] and that of irradiation with X-rays[8] have been studied on normal marrow. This has also been compared with marrow from patients suffering from Addison-Biermer anemia (cf. Chapter 8), polycythemia and leukemia (Chapter 10), and thalassemia (Cooley's anemia) .[7]

Figure 9.5 demonstrates that the mitotic activity of erythroblasts (young red blood cells) is depressed by absence of oxygen. This experiment was carried on in a vessel in which a partial vacuum could be maintained. It is made clear by colchicine that the younger cells, the basophil erythroblasts, are more depressed than the more differentiated ones, which have already some hemoglobin. These important results are to be compared to those mentioned above, on the importance of oxygen for mitosis in the epithelial cells of the mouse's ear.[27] This might have passed entirely unnoticed if a tool had not existed to increase the number of visible mitoses and make counting a simple proposition. It must, however, always be kept in mind that control experiments should be made, for it remains to be proved that colchicine, which has such a wide variety of pharmacological effects (Chapter 7), does not disturb some mitoses more than others. These experiments are, of course, entirely based on the assumption that the alkaloid does no more than "freeze" the mitoses at metaphase.[2, 23, 62]

9.4: Hormone-stimulated Growth

A considerable number of papers have been published following the contributions of Allen, Smith, and Gardner.[2] It is not contemplated to review them all here, even if such a task were possible, for many papers of endocrinological interest do not mention in their titles that colchicine has been used, and it has become impossible to keep up a complete set of references. Table 9.4 gives a summary of some of the work which has been published. It is evident that the sex hormones have been the most studied, partly because their isolation and chemical identification took place in the period im-
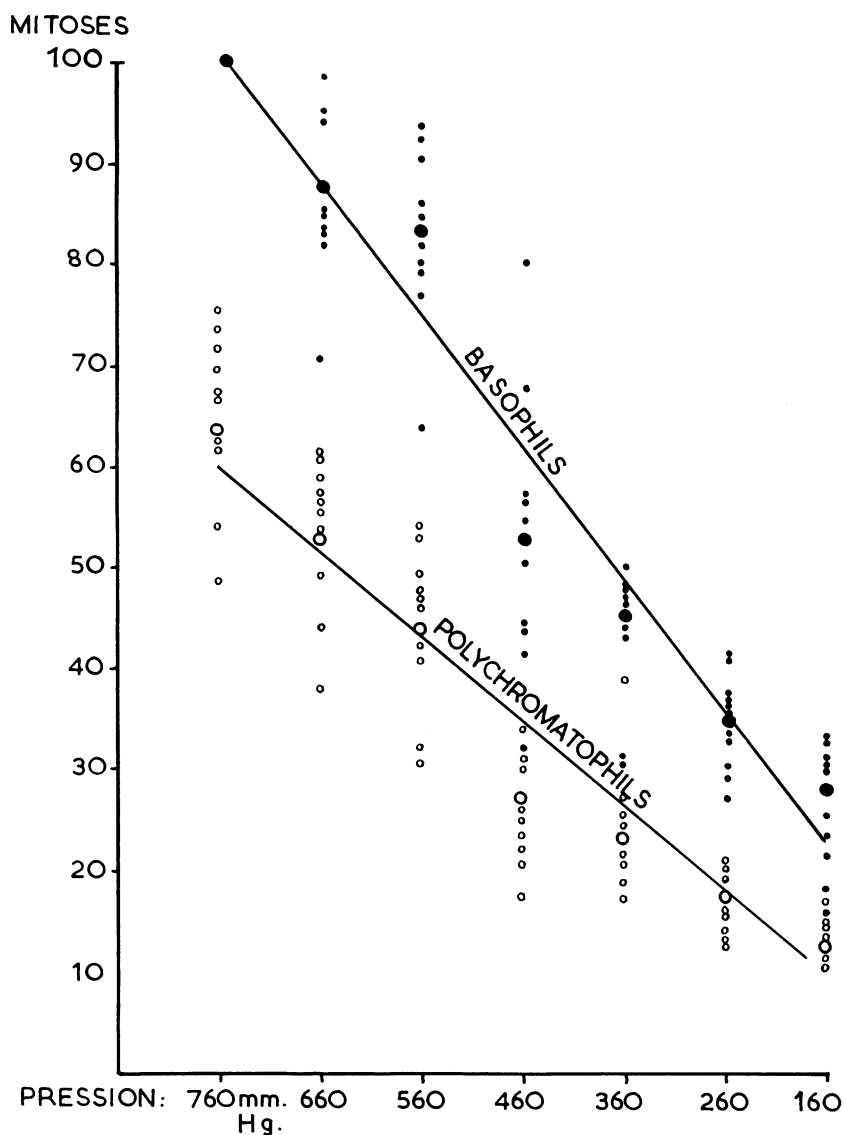
Fig. 9.5—Linear relation between pressure of atmospheric air and mitoses in bone-marrow erythroblasts studied by culture **in vitro**. The results are expressed as percentages of the maximum mitotic rate, i.e., that of basophil erythroblasts at atmospheric pressure. (After Astaldi **et al.**[5])

TABLE 9.4
EXPERIMENTS ON HORMONE-INFLUENCED GROWTH

Hormones	Animal	Receptor Tissue and References
1. *Pituitary hormones*		
gonadotropins	mouse	uterus (muscle)[3]
	rabbit	uterus[30]
	guinea pig	hypophysis[78]
		parathyroid[78]
		adrenals[78]
		testis (interstitial cells)[78]
		seminal vesicles; uterus[12]
	Molge marmorata	prostate[78]
		cloacal epithelium[78]
chorionic gonadotropins	mouse	uterus (glands and muscle)[3]
	guinea pig	testis (interstitial cells)[53]
	rabbit	uterus[30,41]
prolactin	pigeon	crop-sac[58,59,56]
adrenocorticotropic hormone (ACTH) *	rat	adrenal cortex[92]
thyrotropic hormone	rat	thyroid[50]
	guinea pig	thyroid[12,11] †[44]
anterior lobe extract	rat	Langerhans' islets[50,92]
2. *Ovarian hormones*		
a. estrogens ("theelin," estradiol, estrone, etc.)	mouse	vagina[2,91]
		uterus: glands,[91] muscle[2]
		mammary gland[2]
		hypophysis ‡[9,54]
		rectum[91]
		ovary[91]
		skin[24]
		seminal vesicles[10]
		various tissues[24]
	rat	uterus (muscle)[3]
		vagina[73,32]
		hypophysis[55]
		parathyroid[12]
		seminal vesicles[44,67]
		ventral prostate[67,29]
	guinea pig	nipple[89]
		uterus (muscle)[15]
	woman	vagina[79,72]
	Rhodeus amarus	ovipositor[18]
	rabbit	uterus (glands)[28]
		(muscle)[28]
b. progesterone	rat	parathyroid§[12]
		vagina[32]
		prostate[37]

TABLE 9.4 (*continued*)

Hormones	Animal	Receptor Tissue and References
c. pregnane	woman *Rhodeus amarus*	seminal vesicles[37] vagina[79,72] ovipositor[18]
3. *Testicular hormones* (androgens) testosterone, androsterone, androstenediol	mouse rat guinea pig *Rhodeus amarus*	seminal vesicles[65,85,87,39,40] *Id.* transplanted to female[82] prostate[85] seminal vesicles[12,85,44,67,29] prostate[85,29] thyroid (in female)[68] parathyroid (*id.*)[68] adrenal[69] skin epoophoron[48] ovipositor[18]
androstane	*Molge marmorata*	prostate[78] cloacal epithelium[78]
4. *Adrenal cortical hormones* a. desoxycorticosterone	rabbit	uterus[67]
b. cortisone	mouse	skin[27]
c. corticosterone, total extract of cortex	*Rhodeus amarus*	ovipositor[18]
5. *Other hormones* thyroxine	mouse rat	adrenal[47] regenerating kidney[51]
tachysterine	rat	parathyroid[12]
insulin	rat	Langerhans' islets[33]

* Non-purified extract of pituitary.
† Extracted from human urine.
‡ Also experiments with stilbestrol.
§ With estradiol benzoate.

mediately following 1937, when colchicine was taken up as a "fad." Endocrinologists appear to have lost some of their interest in this tool, and this may explain how such important substances as cortisone and ACTH have hardly been tested by colchicine methods. Most of the work was on hormones which stimulated mitosis; cortisone, on the contrary, appears to have an inhibitory action.[24] The usefulness of colchicine in the study of mitotic inhibitors has not yet been fully understood, and further work will undoubtedly demonstrate

that this is a tool for the study of mitotic activity, whether stimulated or depressed. Results reported in Chapter 10 support this opinion.

9.4–1: Pituitary hormones. Prolactine, the hormone stimulating secretion of the mammary gland in mammals, was used in one of the first and most spectacular experiments of this type. In birds, this hormone stimulates the crop-sac. This organ secretes "milk" by a

TABLE 9.5

MITOSIS IN THE CROP-SAC OF THE PIGEON AFTER PROLACTIN STIMULATION
(Colchicine is injected 9 to 11 hours before the animal is killed.)
(After Leblond and Allen[59])

	Number of Animals	Colchicine (*mg.*)	Pro-lactin (*bird units*)	Mitoses per 2000 Cells (Average, Smallest, and Greatest Figures)	Average Mitotic Index
Colchicine controls..	2	0.10–0.25	....	12 (9–15)	1
	6	0.40–0.50	....	46 (8–173)	2
Prolactin controls...	2	....	40	15 (9–21)	1
Prolactin-colchicine..	6	0.10–0.35	40	27 (11–48)	1
	5	0.50	40	534 (210–1075)	26

process which has no relation to that observed in mammals. The bird's "milk" is made of fat-laden cells desquamating from the thickened epithelium of the crop-sac. Table 9.5 shows the increase of the mitotic index for this epithelium in pigeons injected with prolactine and colchicine.[58, 59] In one animal, 53 per cent of all epithelial crop-sac nuclei were found to be in a condition of arrested mitosis. The average increase of the mitotic index is 37-fold, and calculation based on the assumption of arrest only, leads to the result that the pro-lactine-stimulated cells must divide in about 16 minutes. It is not certain that such a calculation is correct, because many factors, for instance cellular differentiation, are involved. Also, from the published photomicrographs[59] it is not evident that the thickness of the control and the colchicinized epithelia are comparable. Whatever the significance of these quantitative estimations may be, colchicine demonstrated clearly that connective tissue cells, and muscular cells of the crop-sac wall also divided under the influence of prolactine. This fact had never been observed.[56, 57]

The thyroid-stimulating hormone, thyrotropin, also increases the cell divisions in the thyroid. This is made much more evident by spindle poisoning. In controls 6.3 mitoses were found per 100 thyroid

vesicles in the guinea pig. This figure was increased to 16.8 by the hormone alone, and to 119 by hormone + colchicine.[12] A method for the detection of increased amounts of this hormone in the urine of patients has been proposed[11] (Table 9.6). A response is positive when more than 4 mitoses per 100 vesicles are detected.[11] Other authors have confirmed these results, but some abnormal responses were attributed to a rhythmic growth response of the thyroid.[50]

The gonadotropic hormones stimulate mitotic growth in many tissues, and this was studied by means of colchicine as early as 1937.[12] In the uterine glands of guinea pigs, colchicine made clear the location of the zones of maximal growth. Action of pituitary hormones on endocrine glands will be considered later. Results of work on pregnant guinea pigs may be mentioned however, because they bring evidence of many, often unsuspected stimulations of mitosis by the increased amount of gonadotropic and other steroid sex hormones during pregnancy.[33] Especially notable is the stimulation

TABLE 9.6

MITOSES IN ONE MICROSCOPIC FIELD IN THE THYROID OF THE GUINEA PIG
(After Bastenie[11])

Substance Injected	Number of Cases	Mitoses
Colchicine alone	. . . .	0.5–1
Anterior lobe extract + colchicine	. . . .	35
Extracts of urine + colchicine:		
myxedema	5	1–7
myxedema after treatment	3	0.1–0.5
hyperthyroidy	3	0.15–0.25
hypothyroidy of pituitary origin	3	0.05–0.4
Froelich's syndrome	2	0.05–0.1
Acromegaly	1	0.2
Other diseases, without thyroid disturbances	3	0.2–0.4

of the parathyroids, exocrine glands of the pancreas, and kidney tubules — changes which would have been unnoticed without colchicine. This important work does not seem to have been pursued so far as colchicine is concerned (Table 9.7).

The absence of publications on the adrenocorticotropic hormone (ACTH) and colchicine has already been mentioned.[24] It is still

TABLE 9.7

MITOTIC INDEX IN ORGANS OF PREGNANT GUINEA PIG

I: without colchicine
II: 9 hours after 0.625 mg./100 g. colchicine
A: embryos less than 5 mm. long
B: embryos from 5 to 15 mm.
C: embryos longer than 15 mm.

(After Cavallero[33])

	Controls		A		B		C	
	I	II	I	II	I	II	I	II
Hypophysis (anterior lobe).....	0	2	5	*17*	1	7	0	6
Thyroid............	0.2	0.3	2	7	1	5	0	3
Parathyroids.........	0	1	1	*14*	0	7	0	2
Adrenal cortex.......	0	0	2	4	0	4	0	1
Adrenal medulla.....	0	0	0	0	0	0	0	0
Langerhans' islets.....	0	1	0.3	*5*	0	0	0	0
Corpus luteum.......	0	. . .	1	1	6	6	..*	5
Kidney.............	0	2	2	8	9.5	5	0.5	*18*
Pancreas (exocrine)...	0	0.5	1	4	0	*10*	0	5
Liver..............	0	0	0	0	0	0	0	0

* The figure given in the original paper has been omitted because of a typographical error which it has not been possible to correct (Cavallero, personal communication).

more remarkable that the growth hormone, somatotropin (STH), has only been studied with the colchicine method in a single paper, which pointed to stimulation of hemopoiesis.[74] This shows that many pathways remain open. The results obtained with other hormones are good evidence that important and unsuspected findings still remain before us.

9.4–2: Sex hormones. These are powerful stimulants of mitotic growth. Some of the results with estrogens have been reported in the first paragraph of this chapter.[2, 93] It was not always realized that estrogens may stimulate growth in other epithelia than those of the genital tract. In his observations on mice, Bullough, using colchicine to detect the increased mitotic activity, demonstrated stimulation in most tissues, including connective tissue.[24] In further experiments, this author has called attention to a remarkable effect of estrogens. Figure 9.6 shows that colchicine increases the mitotic index of the
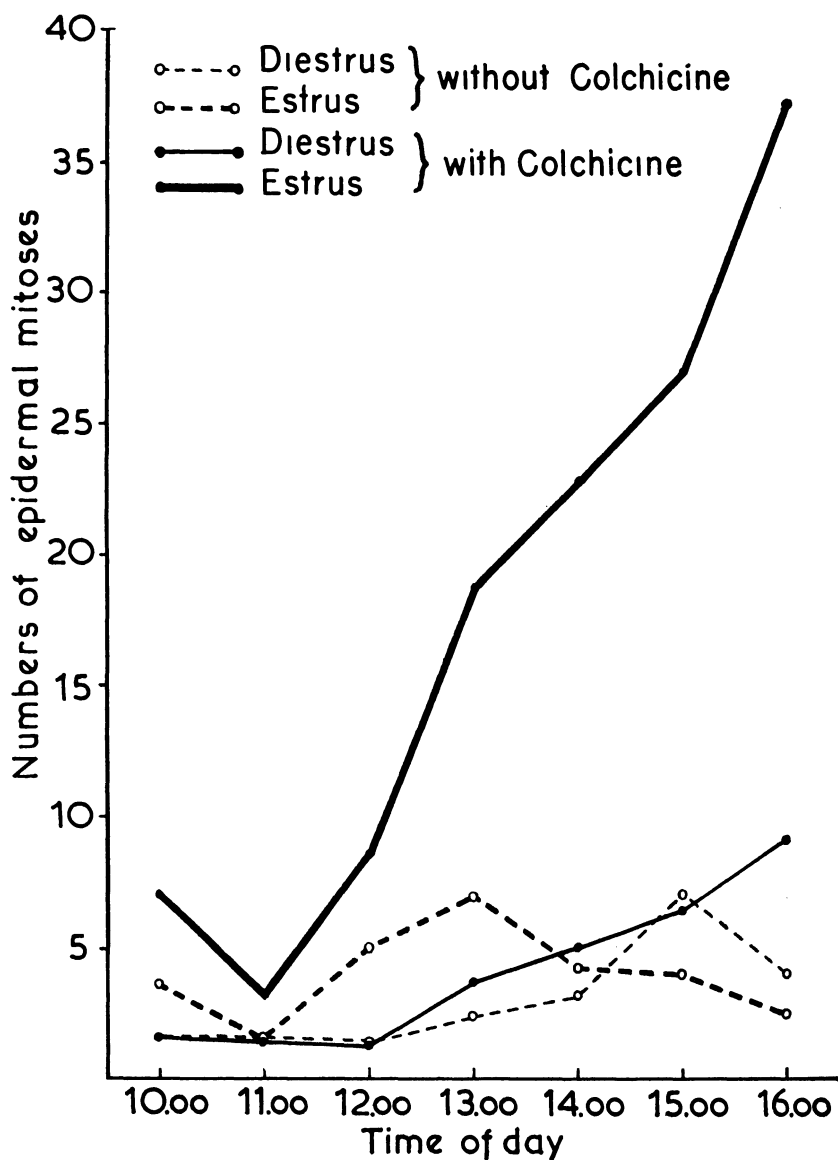
Fig. 9.6—Mitotic activity, as demonstrated by colchicine, in the epidermis of the ear of female mice, in estrus and diestrus. Controls: dotted lines. The far greater increase observed after colchicine during estrus is considered to be an indication that normally epidermal mitoses last longer in diestrus. (Modified, after Bullough, 1950[24])

epidermis of the ear considerably more during estrus than during diestrus. The mitoses were counted hour by hour by clipping small fragments of the ear. This difference can be explained by a shortening of the time taken for one division, from about 2 hours in diestrus to ¾ hour in estrus. This significant result is not discussed; other possible hypotheses are, for instance, synergic action of colchicine and hormone, or changes in the duration of interphase. The alkaloid is simply considered to stop metaphases.[2, 23, 62]

Androgenic hormones, also, stimulate mitotic growth, and the use of colchicine was advocated in 1937 for the study of the changes in the seminal vesicles[85, 65, 12] (Fig. 9.7). The accumulation of arrested mitoses in the prostate or seminal vesicles of castrated mice or rats has been proposed as a test for androgens.[85] In mice, colchicine helped to prove that the prostate is a more sensitive reactor than the seminal vesicles to testosterone.[44] Data about the "explosive" aspect of mitotic stimulation when studied with colchicine in these tissues has been discussed already and presented in Table 9.1.

The quantitative aspects of the seminal vesicle reaction to various androgens and related hormones have been carefully investigated.[39, 40] Figure 9.8 demonstrates how the increased number of mitoses helps to establish the linear relations between the doses of androgen injected and the intensity of the reaction. With other hormones, such as progesterone and estrogens, though the mitotic index may increase, no such relation is found[40] (Fig. 9.9).

Colchicine also brought further evidence that in the female guinea pig, the epoophoron reacted to colchicine like the male epididymis, of which it is the anatomical homolog.[48]

9.4–3: Mitotic stimulation of endocrine glands. Though pituitary hormones play a great part in mitotic stimulation in various organs, the cells of the pituitary may also undergo mitosis under the influence of hormonal stimuli.[9, 55, 71] Colchicine helped to demonstrate that in virgin female rats, ovariectomy did not promote pituitary mitoses.[55] On the contrary, injections of estrogens, natural or synthetic, enlarge the pituitary as a consequence of mitotic growth made evident with colchicine.[55] It has, however, been shown that castration could influence the numbers of c-mitoses of the basophil cells of the anterior lobe of the pituitary.[71] There are no data about the posterior lobe of the organ, which may be an interesting object for future colchicine work.

Several papers deal with mitotic stimulation in the cortical region of the adrenals.[92, 78, 68, 69, 47] In immature female rats, colchicine reveals a stimulation which reaches its maximum 96 hours after an injection of testosterone. At the same time, however, mitotic activity is increased in thyroid, parathyroid, and ovary. This may be evi-
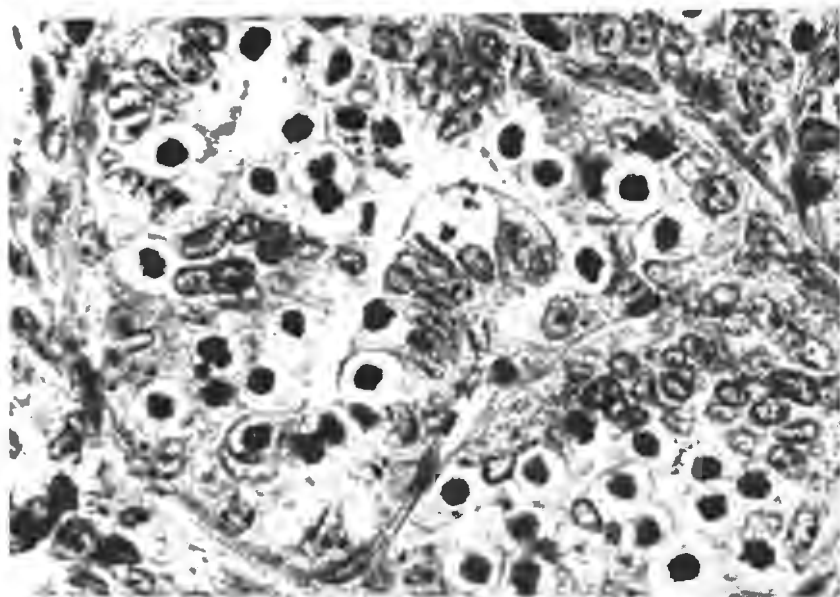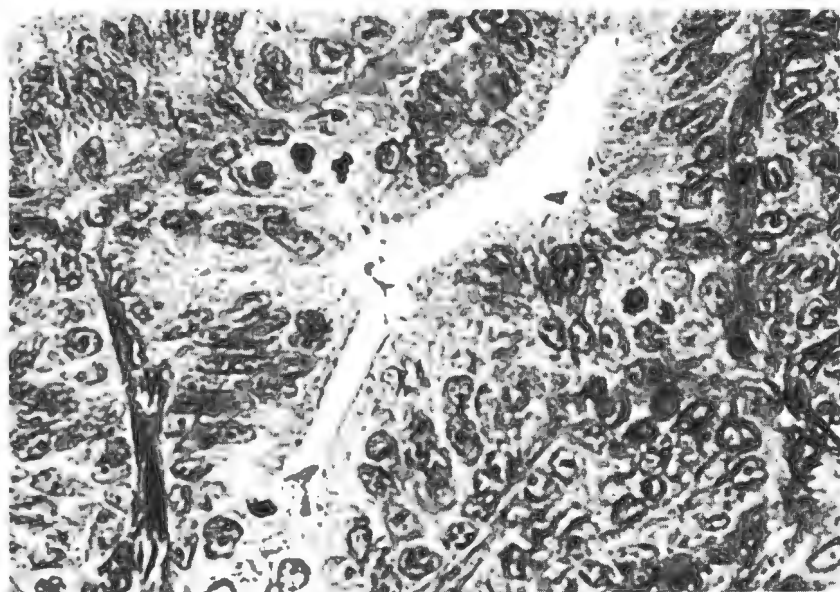
Fig. 9.7—Mitotic stimulation by testosterone propionate in the seminal vesicles. **Above.** Hormone alone. **Below.** Hormone + colchicine. (Original photomicrographs from Bastenie and Zylberszac[12])
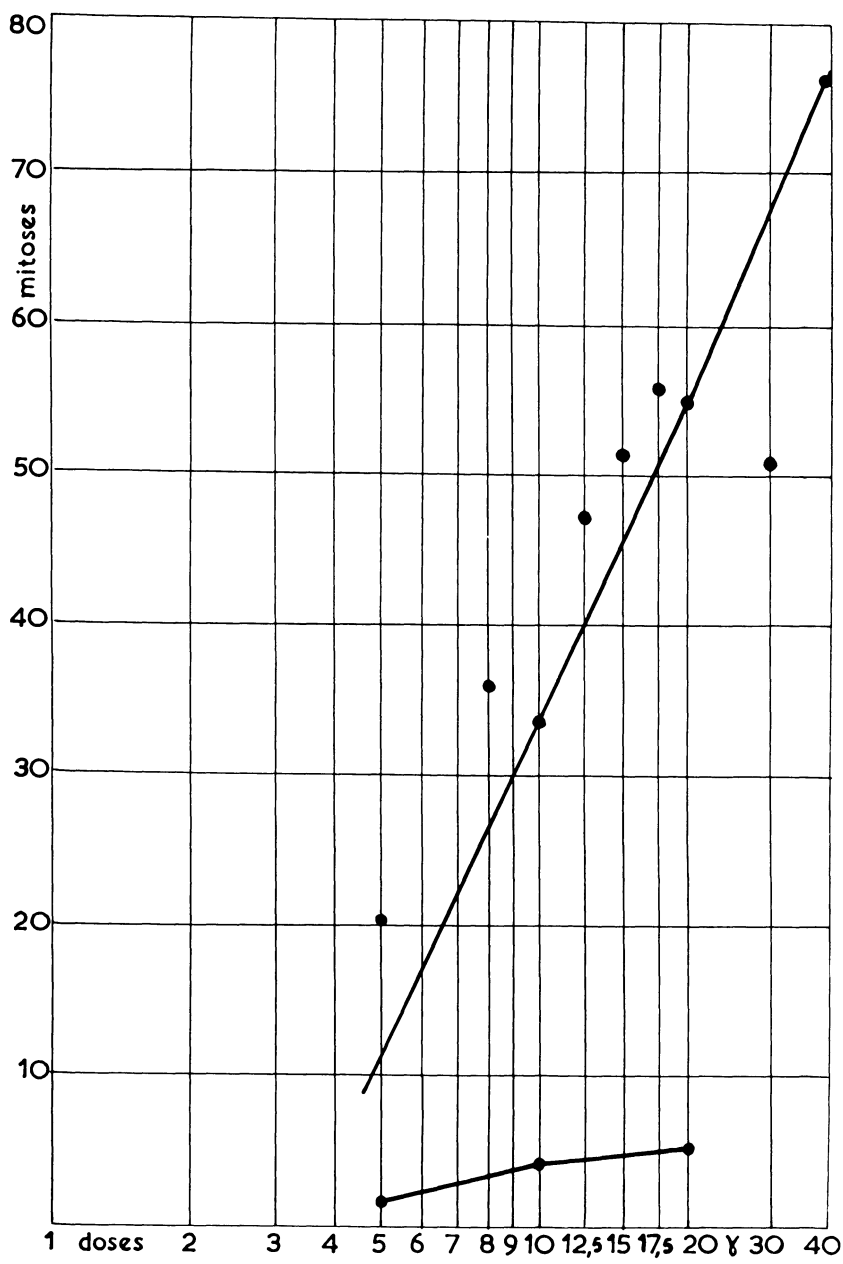
Fig. 9.8—Seminal vesicle test with testosterone propionate. The line (**below**), without colchicine, does not make clear the correlation between number of mitoses and dose. With colchicine, a linear relation is evident (**above**). (After Dirschel et al.[40])
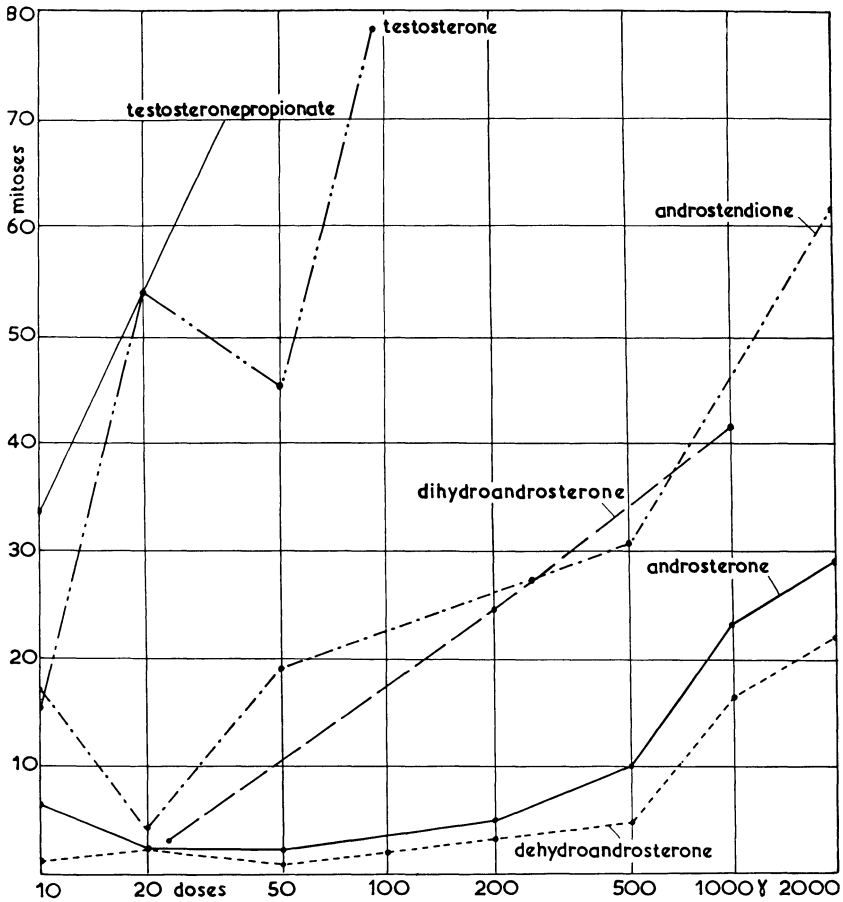
Fig. 9.9—Seminal vesicle test with various androgens. Amplification of the number of visible mitoses by colchicine. (After Dirschel et al.[40])

dence of an indirect action via pituitary stimulation.[68] The same applies probably for the increased mitotic activity detected in the thyroid of female rats injected with testosterone.[69]

The mitotic activity of the parathyroid glands of mammals is usually very low, and is difficult to study; hence, the utility of colchicine. *Folliculin* (estradiol) and progesterone injections in the rat result in the appearance of many mitoses.[12] This effect may be the consequence of hypocalcemia. The contrary, hypercalcemia, probably explains why irradiated tachysterin (Holtz's A.T.10) decreases the parathyroid mitotic activity. Testosterone injections increase mitoses in this organ; this may be an indirect effect mediated by the pituitary.[69]

In the Langerhans' islets of the pancreas, pituitary stimulation [92, 50] and pregnancy increase the number of mitoses, as detected by colchicine.

It is surprising to find no paper dealing with mitotic stimulation in the interstitial (Leydig) cells of the testes. In guinea pigs injected with chorionic gonadotropins, these cells increase in number, but colchicine failed to detect mitoses. It was concluded that the hormone-secreting cells originated from ordinary connective cells.[53] Further work on this tissue is obviously needed.[46]

9.5: Regeneration and Hypertrophy

The problem which was under study in the laboratory of A. P. Dustin, Sr., since about 1920 and which led to the discovery of the properties of colchicine was that of the regulation of growth and mitotic activity in pluricellular animals. In vertebrates, for instance, cell division takes place only in some tissues, and then in an orderly way. While in the adult, nerve cells become incapable of any mitosis, other organs, such as the liver and the kidney, while nearly devoid of any mitotic activity in normal conditions, may grow rapidly by cellular multiplication after surgical excision. In the rodents, and in particular the rat, large portions of the liver may be removed surgically. The remaining cells start to divide at once, and regeneration of the normal liver mass is remarkably rapid.[21] The exact determinism of this cellular growth is unknown. This was one of the first subjects to be studied with the help of colchicine as a tool for a better analysis of mitotic activity.[19, 20, 21, 22] Hence, the work which had been initiated in order to understand better such problems as regenerative growth led indirectly to the discovery of a new tool, colchicine, which was rapidly put to use in several countries.[19, 31, 41, 43] The problems of cellular division in wound healing, which is closely related to regeneration, will be considered in the next section of this chapter. This work deserves special attention, for important results appear to have been often overlooked. Once again, colchicine was taken up with enthusiasm as a new tool; new discoveries were made possible, but only in a few instances was the study pursued long enough to come near a solution of the problems.[51] This field appears today as one of the most promising for future research.

9.5–1: Liver. In the rat, as much as 68 per cent of the liver parenchyma may be removed surgically. After an initial period of edematous swelling lasting about 24 hours, cell division takes place. This type of growth has been extensively studied, for it lends itself to quantitative estimations of the numbers of new cells formed each day.[2] The duration of mitosis was found to be between 48 and 53 minutes. After colchicine, many arrested mitoses are visible. Their

number can be explained on the basis of mitotic arrest.[19, 20, 21] Some show only slight abnormalities, but most are of the exploded type (Fig. 2.5). When up to one-fifth of all the liver cells are in this condition, swollen and their chromosomes dispersed, the liver becomes extremely friable.[22] The various stages of restitution after the injection of colchicine have been described and illustrated in Chapter 2. It is surprising that the regeneration is only slightly slowed down by several injections of the sublethal dose of 50 mg. This has been explained by the fact that the exploded metaphases, after building cells with many micronuclei, regained normal nuclei by the fusion of the micronuclei (Figs. 2.7, 2.8, 2.9). These facts remain rather difficult to understand from a quantitative point of view.

Apart from this work, liver regeneration studied with colchicine has provided some material for counting the chromosomes. This is done readily in the exploded metaphases. Diploid, tetraploid, and octoploid nuclei were observed, a fact which agrees with karyometric data.[35] About the analysis of the differential growth of various liver constituents — liver cells, Kupffer cells, bile canaliculi, blood vessels — hardly anything is known, and there remain ample opportunities for further colchicine research.[83, 64, 75] The biochemical stimulus to mitotic growth after hepatectomy is also unknown; some unpublished results obtained at Brussels indicate that the ligature of bile ducts may increase mitoses, as observed in the liver by the colchicine method.

9.5–2: Kidney. The increase of the volume of one kidney after removal of the other is closely related to regeneration. It proceeds by mitotic growth. This is particularly difficult to analyze in such a complex organ as the kidney, and any tool increasing the number of visible mitoses is most helpful.[41, 43, 31] The great number of mitoses observed in rats injected with 2.5 mg/kg after unilateral nephrectomy and killed 10 hours later is apparent from Table 9.8.

The problems of kidney mitoses in this condition and in other experiments carried on to throw light on the causal factors have been the object of several publications from the Brussels school. After unilateral nephrectomy, the maximal number of mitoses is found during the first four days in the convoluted tubules, then in the glomeruli, and on the seventh day in Henle's loops and the Schweiger-Seidel tubules.[41, 43] No mitoses are to be found in the epithelium of the renal pelvis. Exploded c-mitoses are the most frequent in the convoluted tubes. If a partial nephrectomy is added to the ablation of the other kidney, the remaining tissue shows mitoses in all locations, including the pelvis. Ligation of the ureter, without nephrectomy, also stimulates kidney cells to divide, a fact which may prove of great experimental importance[51] (Fig. 9.10). Another re-

markable result is found when colchicine is injected into animals after one renal artery has been ligated.[43] The ischemic kidney shows a considerable number of mitoses, mainly in the excretory (Schweiger-Seidel) tubules and the pelvis (Fig. 9.11). Similar facts have been observed in kidneys made partly ischemic by the endocrine kidney operation of Selye.[33] The following experiments were aimed

TABLE 9.8

Mitotic Index in the Remaining Kidney of Adult Rats Injected With Colchicine
(After Carnot and May[31])

Days After Unilateral Nephrectomy	Cortex	Medulla		Total
		External Zone	Internal Zone	
Controls...............	3	1.5	0	4.5
3.....................	43.5	13.5	0.5	57.5
8.....................	43	9	1.5	53.5
14....................	18	4	0	22
21....................	18	0	0	18

at finding the possible nature of the mitotic stimulus.[51] The number of renal mitoses after nephrectomy was decreased by injections of thiouracil, a drug which depresses thyroid function. Thyroidectomy, however, did not prevent or retard the increase of size of the remaining kidney in the rat.[51] Thyroxin was nevertheless found to stimulate renal mitoses as much as would a nephrectomy. When this was carried on and thyroxin injected afterwards, the mitotic increase was greater than expected. This may indicate a truly synergic action of the two stimuli. The differences in body weight between controls (nephrectomy alone) and the other rats, and the fact that the mitotic counts were corrected for 100 g. of body weight, make these results difficult to interpret and suggests the need for further research (Table 9.9).

The hypothesis which was put forward following these data was that thyroxin did not act directly on renal tissue, but that the increased protein catabolism resulting from the action of the hormone provided the factor responsible for mitosis.[51] Some substance present in the urine may be suspected since, as mentioned above, ligature of the ureter promotes cell division (Fig. 9.10). However, such mitotic activity is mainly located in the connective tissue of the kidney. An important fact is that unilateral ureter ligation promotes mitosis in
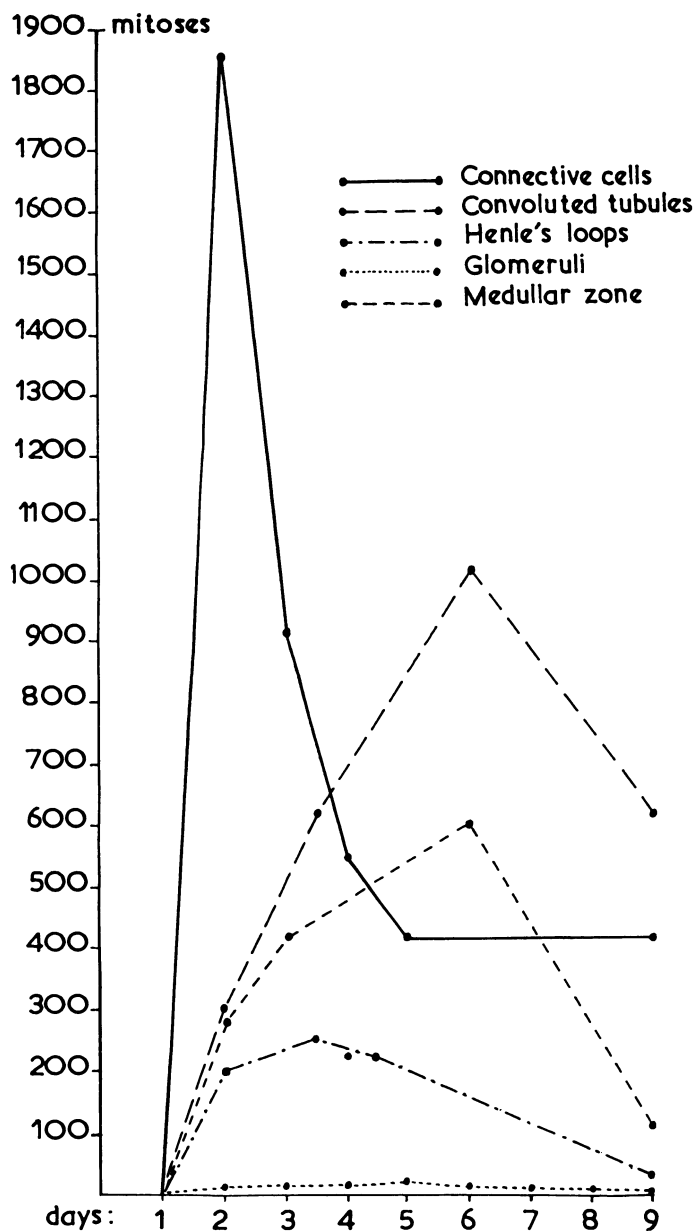
Fig. 9.10—Mitotic activity in the kidney of the rat after ligature of the ureter, studied with the colchicine-technique. (After Herlant[51])
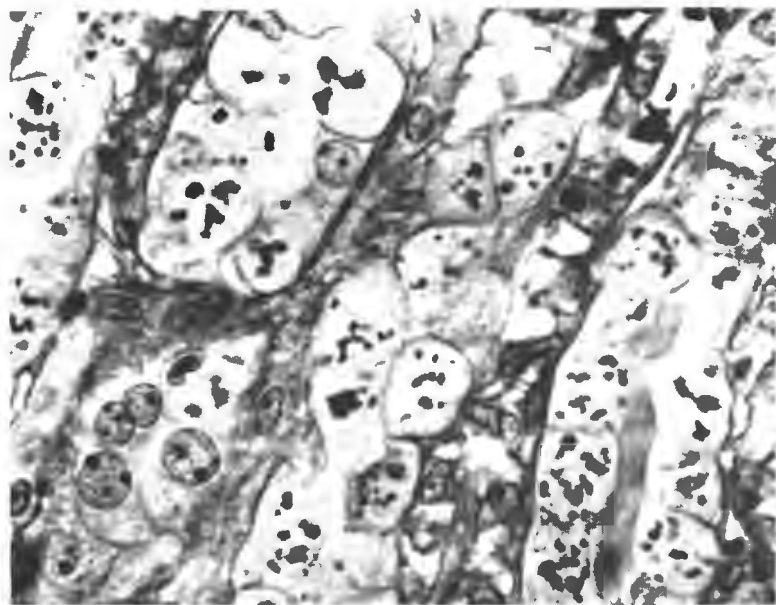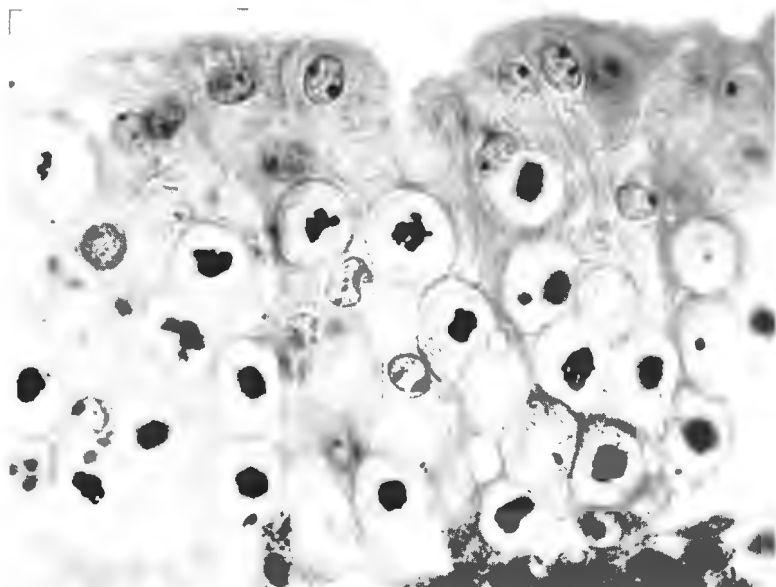
Fig. 9.11—Colchicine-mitoses in the kidney of the rat, 72 hours after ligature of the renal artery. **Above.** Star and ball metaphases with clumped chromosomes in the renal pelvis. **Below.** Exploded metaphases in the tubuli centorti. (A. P. Dustin ond Zylberszcc[41, 48])

the other kidney also; this resembles closely the changes of com-
pensatory hypertrophy (Fig. 9.12). Substances reabsorbed from the
urine may promote division first in the ligated kidney and later
in the other one. Research by other workers has suggested that
xanthopterin or substances of that chemical constitution may initiate
the kidney hypertrophy. The problems are far from being solved,
but the utility of colchicine for the observation of mitotic growth
has been amply demonstrated.

9.5–3: Other organs. The following results give an indication
of the multiple uses of colchicine as a tool. In the Langerhans' islets
of the pancreas, alloxan brings about a selective destruction of the
so-called β-cells, which secrete insulin. Regeneration and mitoses of
these cells are prevented if the animals receive insulin. This proba-
bly acts through a pituitary mechanism, for extracts of the pituitary
gland increase considerably the number of cell divisions in islet re-
generation. Colchicine-mitoses are also observed in the anterior lobe
of the pituitary[33] (Table 9.10). The regeneration of the adrenal
cortex after unilateral adrenalectomy in rats has also benefited from
the use of mitosis arrest.[10] In rats also, colchicine helped to demon-
strate that compensatory hypertrophy of parathyroids after partial
parathyroidectomy does not take place in hypophysectomized ani-
mals[37] and that testosterone inhibited the epithelial mitoses in thymic
regeneration following X-irradiation.[49]

TABLE 9.9

Action of Thyroxin on Renal Hypertrophy After Unilateral Nephrectomy
Number of Mitoses in a Median Section of the Whole Kidney,
9 Hours After Colchicine
(Abridged from Herlant[51])

	Mitoses					
Experiment	Convo- luted Tubules	Henle's Loops	Glo- meruli	Me- dulla	Connec- tive Tissue	Total
1. Unilateral nephrectomy (4 rats) *.........	61–125	8–19	0–3	26–87	44–62	163–210
2. Thyroxin alone (4 rats) †.........	173–252	3–5	0–2	2–5	3–7	186–250
3. Unilateral nephrectomy + thyroxin (7 rats) †.........	315–589	35–65	2–15	25–152	31–132	523–722

* Animals weighing 260–360 gm.
† Six daily doses of 0.25 mg. thyroxin; killed the seventh day after 2 mg/kg col-
chicine. Animals weighing 120–220 gm.

9.5–4: Regeneration in developing animals. The complex actions of the *Colchicum* alkaloid in embryonic development and larval growth have already been reviewed. It is not surprising that in some conditions colchicine may actually inhibit regenerative growth; thus, it could not properly be used as a tool. In *Amblystoma opacum* and *A. punctatum*, 18 to 25 mm. long, limb regeneration was studied
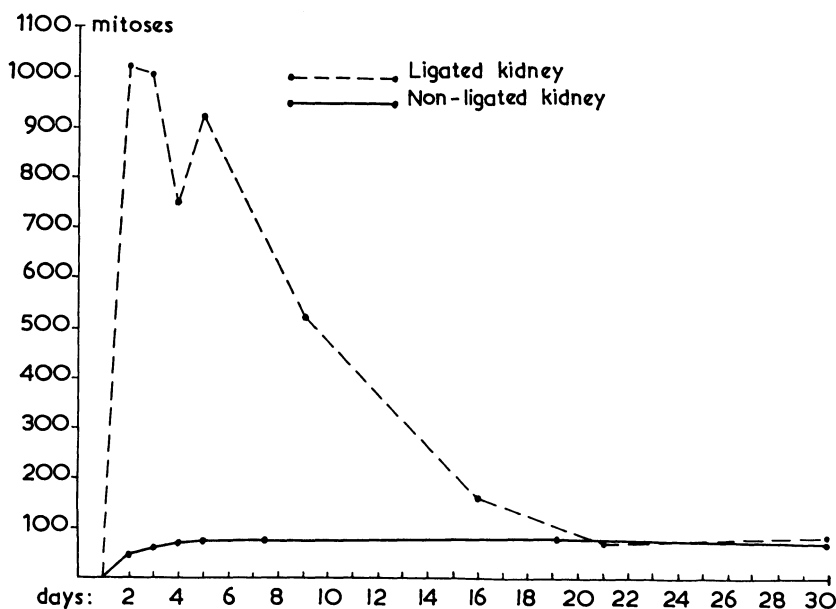

Fig. 9.12—Unilateral ligature of the ureter in a rat. Mitoses in ligated and non-ligated kidney, detected by the colchicine-method. (After Herlant[51])

when the larvae were placed in 1:1000 or 1:5000 solutions of colchicine. If this was done at the moment of amputation, all regeneration was suppressed. Various degrees of inhibition of the limb-blastema formation and of further differentiation, according to the length of the colchicine treatment, were described.[86]

The regenerating tail of tadpoles of *Xenopus laevis* reacts similarly.[63] In very dilute solutions of colchicine, this material provided some results which appeared to indicate not only that mitoses were arrested at metaphase but that a true mitotic stimulation existed. Figure 9.13 shows that in control animals the number of mitoses is quite small. If colchicine is assumed to have only a metaphasic arresting action, it is possible to calculate the number of mitoses which should be observed at various intervals, for the duration of mitosis has been observed and calculated in *Xenopus* (Chapter 3). Figure 9.13 indicates that many more mitoses are found than expected, and
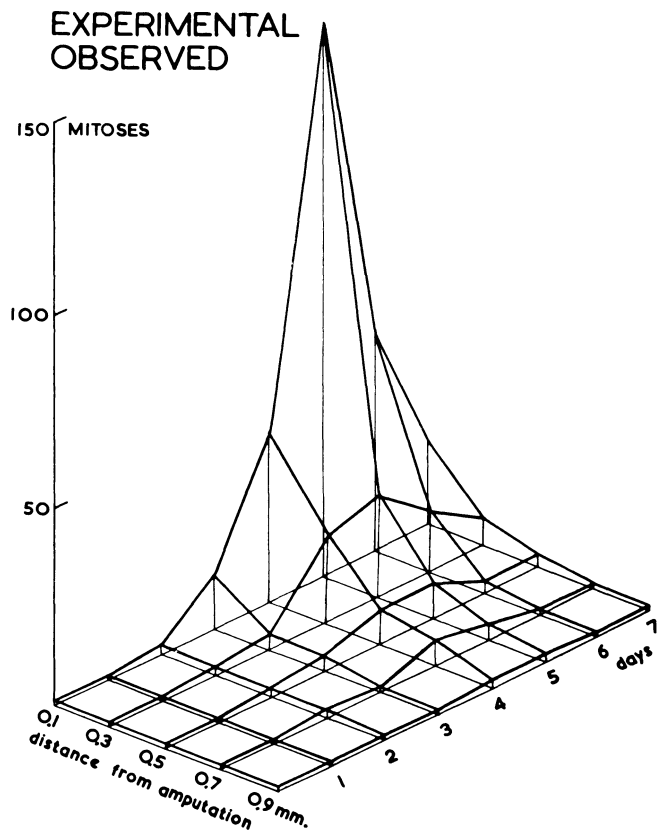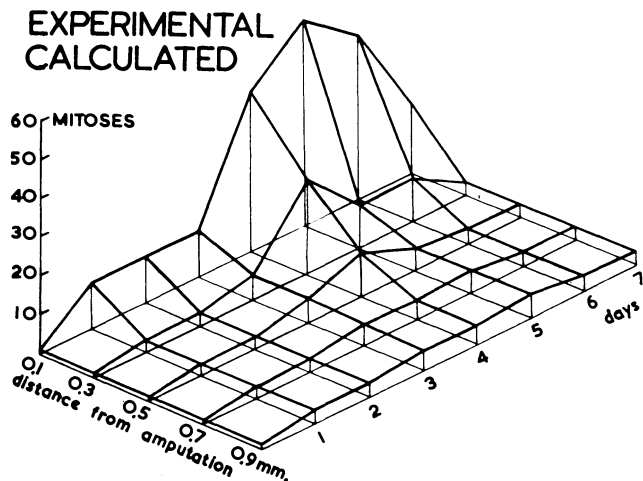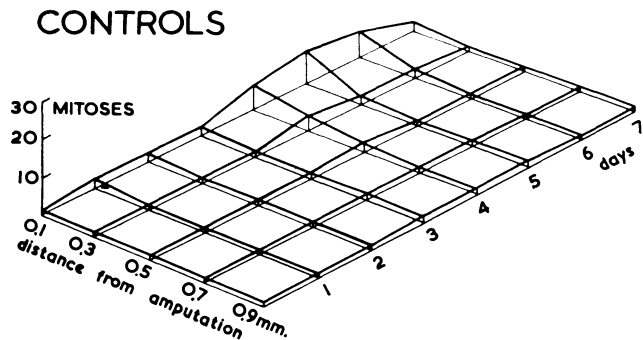
Fig. 9.13—Analysis of the mitotic increase in the regenerating tail of **Xenopus laevis**, in relation to time after amputation and distance from wound. The "calculated" number of mitosis is based on data about the duration of one cell-division in this material, and on the theory that colchicine does no more than "arrest" metaphases. The observed data bring evidence of a considerable increase, close to the wound, on the fifth day. (Slightly modified, from Lüscher[68])

that instead of a gradual rise, there is a steep increase on the fifth day. However, the experimental conditions are complex and stimuli from other growth-promoting substances cannot be excluded. These data with those given in Section 9.2 comprise the best evidence to date of possible mitotic stimulation of animal cells by colchicine.

In *Xenopus,* a short treatment, one hour in a 1:2000 solution, may completely inhibit growth. However, regeneration often proceeds normally during the first three days after this "colchicine shock" because cellular migration is not disturbed. On the fifth day, on the contrary, when divisions should be taking place, regeneration was completely inhibited (Fig. 9.14). Some pharmacological conclusions are important to mention; they are the results of an extensive series of experiments on this favorable material. Colchicine was demon-

TABLE 9.10

INFLUENCE OF ALLOXAN DIABETES ON PANCREATIC, PITUITARY, AND SUPRARENAL
MITOSES; INHIBITION BY INSULIN; STIMULATION BY PITUITARY EXTRACTS

I: rats injected with 150 mg/kg alloxan
II: *id.* + 10 to 20 units insulin per day
III: *id.* + pituitary extract (about 32 mg. dry powder per day)
(After Cavallero[33])

	Mitoses								
	Langerhans' Islets			Anterior Lobe of Hypophysis			Adrenal Medulla		
Days	I	II	III	I	II	III	I	II	III
1....	8	0	3	15	15	24	2	4	1
2....	7	1	24	24	19	16	2	7	3
3....	44	2	132	54	2	64	1	0	0
4....	81	0	185	81	9	164	3	2	0
5....	31	1	86	15	14	12	0	9	0
9....	2	0	8	27	8	10	0	0	0
12....	7	1	7	27	22	8	0	7	2

strated to act locally, for no inhibition was observed when only the anterior part of the larva was immersed in the solution. This is also evidenced by the absence of inhibition if colchicine is applied to another wound close to the amputation. Experiments in which the tail blastema was amputated and growth resumed, demonstrated that colchicine did not penetrate more than 2 mm. from the wound. These also showed that colchicine was fixed in the tissues of the wound for

at least three days. Such a fixation of the alkaloid in tissues has not been described in pharmacological work (Chapter 7). The inhibition of regeneration was clearly the consequence of a great number of the mitoses, sometimes up to 70 per cent, being destroyed after a prolonged period of metaphase arrest (cf. Chapter 3).[63] Similar results have been reported in *Rana temporaria* tadpoles. The local
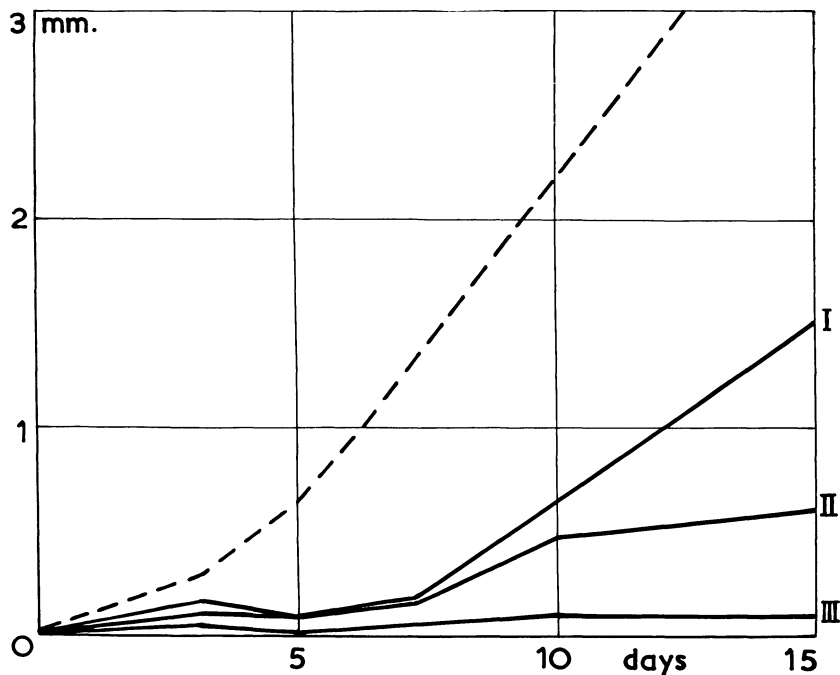

Fig. 9.14—Inhibition of the regeneration in the tail of **Xenopus laevis** after a short treatment with colchicine. Dotted line: normal growth curve. I. Inhibition of regeneration for more than 5 days, then resumed growth. II, III. Strong and persistent inhibition of growth. (After Lehmann et al. 1945, and Lüscher[63])

application of a 1:500 *M* solution of colchicine for only 20 minutes inhibits the regenerative growth of the tail, but has no influence on the growth of the tadpole.[13]

These facts, apart from the conclusion that colchicine is not always a harmless "tool," indicate a remarkable property of the alkaloid of becoming fixed in some tissues. This is surprising for a substance soluble both in water and in lipids. Pharmacologists should pay attention to this possibility, for instance in the analysis of the action of colchicine on muscle and brain. Nearly all data available on colchicine metabolism in warm-blooded animals contradict this

idea of a fixation of the alkaloid. One of the purposes of this book is being fulfilled whenever similar contradictions between work done in widely separated fields of research are brought to light.

9.6: Wound Healing

The histological changes found in wounds after injections of colchicine were some of the most surprising observed by A. P. Dustin, Sr.[42] They appeared to give good support to the theory that a true mitotic excitation followed the injection of the alkaloid. Experiments were performed in rats. Two parallel incisions were made in the dorsal skin, and aleurone grains inserted as an irritant in the wounds before suturing. One of the scars was removed as a control at the time colchicine was injected. The dose was 1.25 mg/kg and the animals were killed 9 hours later. This method made available some new facts about wound healing and the formation of granulation tissue near the aleurone grains. The endothelial cells are the first to divide. Extraordinary pictures of capillaries with up to 10 c-mitoses in a single section were observed. These cells appeared swollen. The rapid mitotic growth was not noticeable without the use of the colchicine tool.[42]

In nerve regeneration, the alkaloid, by increasing the numbers of mitoses, makes clear that their repartition is different on both sides of a section. This may result from the influence of the disintegration products of myelin on the division of the Schwann cells (Fig. 9.15).[36]
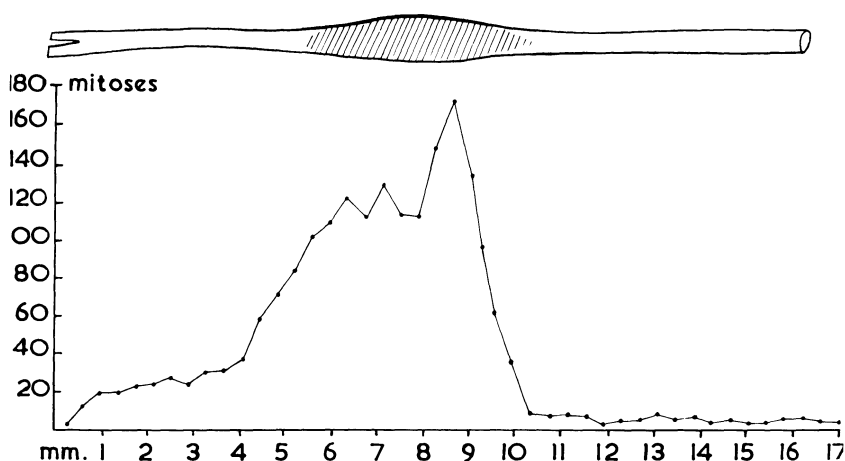

Fig. 9.15—Colchicine-mitoses in a regenerating nerve of the rat. The shaded zone is that of cicatrisation following sectioning. There are more mitoses in the Schwann cells in the peripheric end, at left, than in the central part of the nerve. (After Delcourt[36])

Bone repair has been studied in rabbits.[17] The tibia was cut transversely, without damaging the periosteum otherwise than locally. Mitoses were counted from day to day, the animals being killed 9 to 10 hours after 0.625 mg/kg of colchicine. The amplification of the mitotic changes made estimations of relative growth far easier than in control animals (Fig. 9.16).
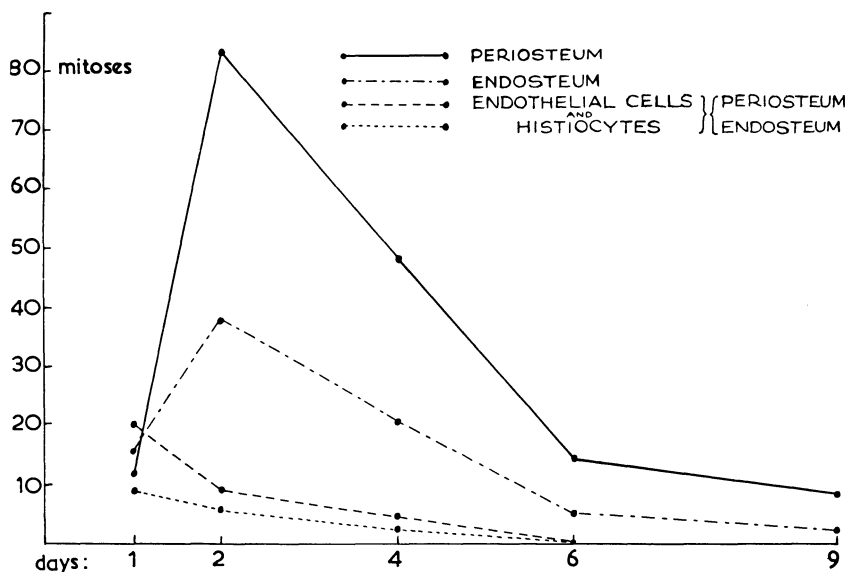

Fig. 9.16—Repartition of mitoses during bone repair, studied after injection of colchicine. (After Borghetti and Parini[17])

These few papers have studied only some limited aspects of healing and inflammatory reactions. Here again, large fields remain open for investigation, and it is surprising that more work has not been completed.

9.7: The Action of Chemicals on Mitotic Growth

Few papers have been published in this section, a surprising fact, for colchicine could no doubt help in the study of many substances affecting growth. In work on vitamins, for instance, many experiments could be imagined. Some results with folic acid antagonists will be mentioned in Chapter 10.

The possibilities of finding new facts is illustrated by the following experiments: Young rats were intoxicated with carbon tetrachloride and studied at various intervals by a routine colchicine technique (Fig. 9.17). Arrested mitoses were observed in the liver cells and in
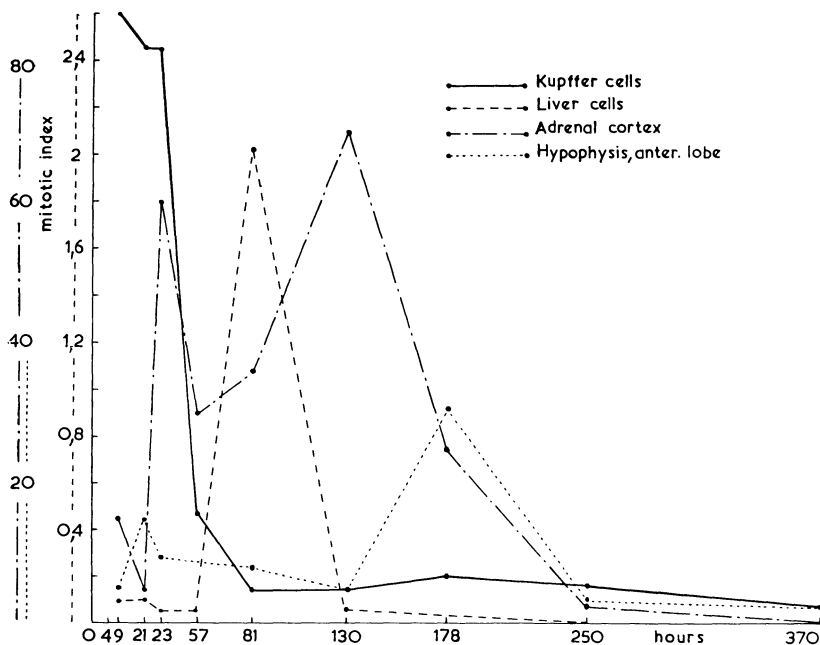
Fig. 9.17—Mitoses in liver and endocrine glands during experimental carbon tetrachloride poisoning, detected by the colchicine-method. (After Cavallero[33])

the Kupffer cells in relation with the progressive cirrhotic changes on the liver. No mitoses were observed in bile ducts, though the number of these apparently increased.[33] After 15 inhalations of carbon tetrachloride, an increased number of reticulo-endothelial mitoses could be observed in the spleen. A systematic study of the endocrine glands revealed evidence of mitotic stimulation in the adrenal cortex, the pituitary, and later, the adrenal medullary zone. These divisions do not appear to be related to local damage, and may be an evidence of a pituitary stimulus arising from "stress" (cf. Chapter 7).

Some work on the mitotic stimulation in the thyroid of rats injected with thiouracil may be mentioned here.[70, 45] The stimulus for cell division is not, however, the chemical itself, but the secretion of the thyrotropic hormone by the pituitary, as mentioned in Subsection 9.4–1. Colchicine has also helped to study, in experiments of this type, the mitotic changes which take place in the pituitary.[16]

Results obtained in young rabbits on the influence of thyroidectomy and thiouracil on healing of cornea wounds are important to consider under this heading, for they throw light on some difficulties of interpretation.[45] Doses of 5 mg/kg of colchicine were injected 4 hours before killing the animals. The results are summarized in

Table 9.11. It is evident that the mitotic index is more depressed by thiouracil than by thyroidectomy, but it seems surprising that this fact is not at all noticeable without colchicine, thiouracil-injected animals having a slightly higher mitotic count than the controls. The authors think that the count after thiouracil results from a double effect, i.e., a decrease of the mitotic rate, which would decrease the mitotic index, and a simultaneous lengthening of the duration of mitosis, which would have the opposite effect.

TABLE 9.11

CORNEAL MITOTIC COUNTS IN A RABBIT

(After Fleischmann and Breckler[45])

	Without Colchicine	With Colchicine
Controls.................	92 ± 35	393 ± 59
Thiouracil-treated........	100 ± 17	168 ± 42
Thyroidectomized.........		228 ± 41

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