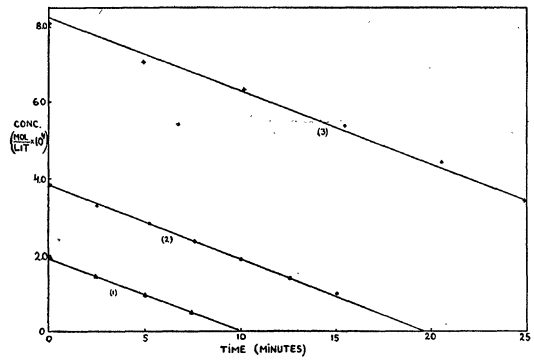


FIG. 3. Relationship between time and the quantity of non-hydrolyzed procaine in samples of various concentrations.

that the reaction rates were equal in all samples. This means that the enzymatic hydrolysis of procaine and chloroprocaine is a zeroth order reaction. The reaction rate constants obtained for three different plasma samples are given in Table 4. As can be seen from this table, chloroprocaine is hydrolyzed about four times faster than procaine.

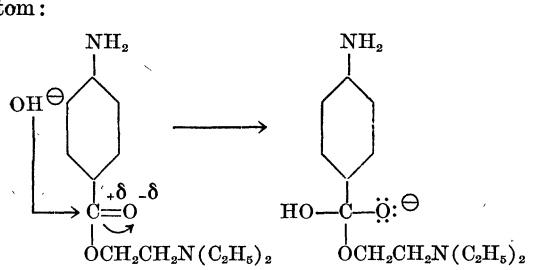
TABLE 4

THE REACTION RATE CONSTANTS (K) OF THE ENZYMATIC HYDROLYSIS OF PROCAINE AND CHLOROPROCAINE AT 37° C

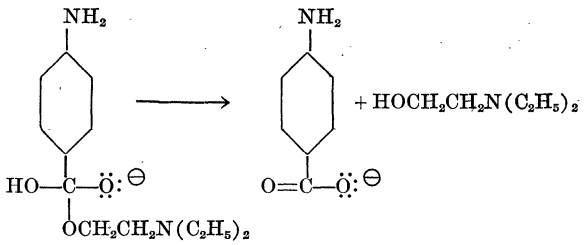
	K in moles/l × min		
	Exp 1	Exp 2	Exp 3
Procaine	2.58×10^{-5}	2.41×10^{-5}	2.45×10^{-5}
Chloroprocaine	1.03×10^{-4}	0.92×10^{-4}	1.04×10^{-4}
$\frac{K_{\text{chloroprocaine}}}{K_{\text{procaine}}}$	3.99	3.83	4.24

Although the mechanism of the alkaline and the enzymatic hydrolysis is different, it is of interest that the ratio of the reaction rate constants of chloroprocaine and procaine hydrolysis in both cases is not only of the same order of magnitude, but actually very close.

On the basis of purely theoretical considerations it was not definitely predictable what effect the substitution of a chlorine atom in the benzene ring would have on the speed of hydrolysis of the procaine molecule. The positive center at which the hydroxyl ion attack should be expected is the carbonyl carbon atom:



The decomposition of this complex will then proceed as follows:



Since the rate-determining step in the above reaction chain is the attachment of the hydroxyl ion to the carbonyl carbon atom, any electrophilic substituents in the benzene ring, which withdraw electrons from the carbonyl carbon atom, will facilitate this reaction and speed up the hydrolysis. The substitution of chlorine conceivably can have one of two effects: It can either exert its electron-attracting permanent polarization effect, or its electron-releasing resonance effect can be predominant. Our experimental findings indicate that when chlorine is substituted in the procaine molecule, the first of these two possibilities is prevalent.

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The Oncological Aspect of the "Immunity" of *Colchicum* to Colchicine

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In the course of his early colchicine work, the senior author came to the conclusion (*1*), as other workers had (*2, 3*), that *Colchicum autumnale* was totally resistant to the toxic, stathmokinetic, and oncogenic actions of colchicine.

Since then, this immunity has been seriously questioned by Cornman (*4*), who elicited typical colchicine mitoses in the roots of *Colchicum* by using higher concentrations of the drug than previous investigators. Levan and Steinegger (*5*) have, however, argued that the striking results obtained by the American author were not due to the alkaloid, but to the chloroform of crystallization in the drug.

These findings have revived old problems and have raised new ones that seem worth investigating—this so much the more so as in the references at our disposal we have found information only on the responses of the roots, whereas previous experiments of Havas[2] have furnished ample indications of the divergent oncogenic and growth effects of extracts of the various organs of *Colchicum*. If different organs of

[1] CNRS and IUBS—Unesco fellow for 1950–51. (Died June 9, 1951.)

[2] Observations and photographic records in press.

the plant yield extracts of different chemistry, it seems logical to expect differences in responses of those organs. On account of their sensitivity the pollen tubes appeared to be the most conveniently adapted to the present preliminary test.

About 4,500 pollen grains of *Colchicum autumnale* L. were germinated under sterile conditions in watch glasses, placed in Petri dishes, and kept at room temperature. The culture solution was composed of 1 g gelatin and 5 g sucrose dissolved in 100 ml tap water. To this, with the exception of the controls, either Merck's crystalline colchicine, containing 12.5% chloroform (hereinafter called colchicine *b*), or pure chloroform (Fr. Ph.) was added in the concentrations shown in Table 1. The culture media were heat-sterilized (according to Tyndall's method) *before* adding the above substances, with the exception of a supplementary colchicine series in three concentrations, in which the order of the process was reversed. As a consequence of this treatment practically all the chloroform content of such solutions (hereinafter called colchicine *a*) was evaporated. The respective concentrations of pure chloroform were calculated to correspond to the full chloroform content (12.5%) of the respective solutions of colchicine *b*. The rate of germination was recorded, and the pollen tubes were measured and drawn to scale 6 times in the course of each of the experiments. Table 1 shows three of these readings.

TABLE 1*

	No. pollen grains	2 hr			14 hr			27 hr				
		G	*L*	*M*	*G*	*L*	*M*	*G*	*L*	*M*	*T*	*S*
Control	600	10	22	32	75	504	1,110	90	945	1,480	0	—
Colchicine *b* 2%	300	0	—	—	0	—	—	0	—	—	—	—
" *a* 1%	210	5	11	14	6	48	200	9	110	270	72	+++++
" *b* 1%	300	3	9	11	5	42	175	7	95	310	80	+++++
Chloroform 0.125%	300	3	18	50	85	435	1,250	87	820	2,025	0	—
Colchicine *a* 0.4%	210	24	12	17	36	69	221	72	108	695	38	++
" *b* 0.4%	300	29	9	13	40	56	150	76	115	759	42	++
Chloroform 0.05%	300	5	14	55	12	105	680	15	616	1,274	25	++
Colchicine *a* 0.1%	210	25	9	15	60	106	255	80	645	1,200	12	+
" *b* 0.1%	300	35	8	12	55	95	280	74	680	1,156	10	+
Chloroform 0.0125%	300	13	20	35	80	480	872	93	880	1,436	0	—
Colchicine *b* 0.025%	300	45	12	15	63	560	865	88	1,002	1,298	0	—
Chloroform 0.0031%	300	15	25	39	81	578	1,220	88	840	1,660	0	—
Colchicine *b* 0.0066%	300	12	20	55	70	680	1,340	85	1,170	1,515	0	—
Chloroform 0.00093%	300	11	19	34	74	600	1,040	93	795	1,500	0	—

* Averages of two experiments, one of which is in duplicate. *G*, germination %; *L*, average length of pollen tubes (μ); *M*, maximum length attained by the longest of the pollen tubes; *T*, number tumors/100 pollen tubes; *S*, relative size of tumors.

The typical effect of colchicine on any germinating pollen grain includes a decreased longitudinal growth of the tube, twisting of the tube, and swelling at the tip. This swelling is apparently identical with the swelling of cells that form tumors in colchicine-treated roots, and is therefore considered an oncogenic effect. The quantitative gradations of effect are recorded in Table 1. The last two columns reveal the significant fact that, although 1% colchicine (both *a* and *b*) induced in the pollen tubes very marked oncogenic effects both from a quantitative and a qualitative point of view, the same concentration of pure chloroform (0.125%) as that contained in the 1% solution of colchicine *b* displayed absolutely none. Paradoxically enough, a lower concentration (0.05%) of pure chloroform, corresponding to the chloroform of crystallization in 0.4% colchicine *b*, produced typical colchicine tumors. These, although significantly less numerous than those induced by 0.4% colchicine *a* or *b*, equaled them in size. As Table 1 shows, none of the other concentrations of pure chloroform had oncogenic effects. In colchicine *a* and *b* the threshold of this activity was 0.1%. Although very few typical club-shaped tumefactions were noticed in this concentration, the pollen tubes were distinctly thicker than those of the controls, and many of them twisted into concentric convolutions. Chloroform at 0.0125%, corresponding to the concentration in 0.1% colchicine *b*, did not show even these anomalies.

As regards the elongation of the pollen tubes, Table 1 shows that 1% and 0.4% colchicine (*a* and *b*) had very marked inhibitory effects, and that even 0.1% depressed it quite considerably as compared with the controls. The nononcogenic concentrations of the drug had no such influence. It is interesting to note that, whereas 1% colchicine *b* reduced the average length of the pollen tubes by about 89%, pure chloroform in the same concentration (0.125%) as the chloroform of crystallization present in 1% colchicine *b* reduced it only to the extent of about 14%. So far, these observations are in keeping with the general rule that elongation antagonizes the formation of colchicine tumors (*6*). On the other hand, in the 0.05% solution of pure chloroform (equal to the chloroform content of 0.4% colchicine *b*), the largest tumor (diam, 60 μ) was precisely produced on one of the longest (1,128 μ) pollen tubes, whereas in colchicine *b*, the largest

intumescence (39 μ) was exhibited on a tube not exceeding 144 μ. Although not as extreme, similar observations were by no means exceptional. It may further be mentioned that, although not oncogenic, the weaker concentrations of chloroform had a tendency to depress the average length of the pollen tubes. As opposed to the effects of colchicine *a* and *b* on tumor growth vs. elongation, those of chloroform were in consequence far from being consistent.

As can be seen, 2% colchicine *b* prevented germination altogether. In the other concentrations the percentage of germination of the pollen grains was in fair agreement with the length attained by the pollen tubes and in inverse relation to the oncogenic potency and the toxicity of the various concentrations of the drugs. Chloroform at 0.125% was once more an exception to this rule. Some of the very considerable divergency in the rate of germination at the very beginning of the experiment may be ascribed to the chance distribution of the large (46 μ–54 μ) and small (30 μ–35 μ) pollen grains, for, as was empirically demonstrated, the former germinated much earlier than the latter. However, such sources of error were, as can be seen, automatically eliminated by the end of each experiment when maximum growth was attained.

Although in most plants the threshold of the oncogenic influence of colchicine upon roots is higher than the threshold value of its stathmokinetic action, the pollen tubes of *Colchicum* were found to be 10–25 times more sensitive to the oncogenic effects of the drug than were the roots to its stathmokinetic activity, according to the findings of Cornman. Yet, when compared with the reactivity of other plants—e.g., wheat, in which germination and growth are completely stopped at 0.4% and tumors are found at 0.0075%—even the pollen tubes of *Colchicum* were found to display a marked resistance to the alkaloid.

In view of the practically identical oncogenic action of colchicine plus chloroform of crystallization and colchicine from which the chloroform was removed, previous axiomatic assertions (*2, 3*), including that of the senior author (*1*), regarding the total immunity of the plant to the alkaloid can hardly be maintained. The findings of Cornman are thus complemented and implicitly confirmed.

Production of pollen tube tumors in *Colchicum* confirms the oncogenic action of chloroform reported by Steinegger and Levan (*7*) for *Allium*. Our experiments do not, however, substantiate a generalization of the thesis of Levan and Steinegger, according to which the action of the alkaloid in *Colchicum* is to be ascribed to the chloroform of crystallization in the drug. In fact, the above-mentioned inversion of the logically inferable respective growth effects and oncogenic potency of 0.05% vs. 0.1% chloroform, as well as the remarkable difference in the length of tubes with colchicine- vs. chloroform-induced swellings, seem to justify the hypothesis, suggested by Cornman, that different mechanisms are operating.

It is readily admitted that, as they now stand, our investigations emphasize rather than resolve the enigmas associated with the complex problem of immunity. Nevertheless, it is hoped that by confirming, in refractory material such as *Colchicum*, the existence of different oncogenic sensitivities and reactivities in different organs of the same organism, search for their active principle will be encouraged. Given the chemotherapeutic implications of the oncogenic (*1, 8, 9*) and oncolytic (*10, 11*) actions of colchicine in both plants *and* animals, it may not be too far-fetched to recall that also among the animals many were thought resistant to various carcinogens, including colchicine, until the appropriate site of administration had been discovered.

Addendum: According to a personal communication from Dr. Cornman, received when this article was ready for press, the colchicine used by him contained no chloroform. The susceptibility of *Colchicum* to colchicine is thus mutually confirmed.

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Changes in Biliary Cholesterol in Abnormal Thyroid States[1, 2]

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It has long been realized that an inverse relationship exists between plasma cholesterol concentration and the level of thyroid activity (*1*). In general, hyperthyroidism is associated with a decreased plasma cholesterol level and hypothyroidism with an elevated concentration, although these changes are not always striking (*2*). The mechanism underlying this relationship remains unexplained. Recent studies from this laboratory (*3–5*) suggested to us the possibility that knowledge of the bile cholesterol content in abnormal thyroid states might help to clarify this important problem.

Two separate series of male Long-Evans rats, aged 8 and 13 weeks, respectively, were studied. Each series consisted of three groups. One group (control) of the first series was fed a stock diet for 28 days; the sec-

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[3] Fellow of the American Heart Association.

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