some adverse effect due to streptomycin, but as the concentration of PAS in the medium was increased, the detrimental effect of streptomycin was diminished. When the PAS concentration was increased to levels that inhibited growth (250×10^{-8} moles or more per ml) the effect of streptomycin was reversed and growth of the organisms was enhanced. At the highest concentration of PAS (1000×10^{-8} moles per ml), growth occurred only in a zone immediately around the cylinders from which streptomycin was diffusing.

These results suggest that in this streptomycin-resistant strain of *E. coli* there is an antagonistic relation between PAS and streptomycin and that when the concentration of PAS is sufficiently high to partially or completely block some process essential for growth of the organisms, the resistant strain is able to metabolize streptomycin in such a way as to by-pass the blocked reaction. Results obtained by permitting PAS and streptomycin to diffuse simultaneously from cylinders placed on seeded plates with no PAS in the medium ruled out the possibility that either PAS or streptomycin was chemically inactivated when the two drugs were mixed. The biochemical implications of these observations correlate well with the results published by MASSART[1] to the effect that streptomycin, which by itself does not affect yeast, may relieve the inhibition of respiration caused in that test organism by trypaflavine or other agents. It should be recalled that the effect of streptomycin or of PAS depends markedly on the physico-chemical conditions prevailing in the solutions[2], and notably on the presence of certain cations or anions[3]. Therefore, further interpretation and development of our results may be expected from further research on the relationship between physico-chemical phenomena in solutions of PAS and streptomycin and the responses they elicit in test organisms.

Our results obtained *in vitro* with *Escherichia coli* provide experimental support for the clinical view that PAS therapy should be instituted concomitantly with streptomycin therapy and that combined PAS-streptomycin therapy should never be started after preliminary treatment with streptomycin alone[4]. The same clinical precaution seems to apply to other diseases that are amenable to streptomycin therapy.

We wish to acknowledge the courtesy of the following firms which supplied streptomycin and/or *para*-aminosalicylic acid for this work: Barnes-Hind Laboratories, San Francisco, California; Cutter Laboratories, Berkeley, California; Heyden Chemical Corp., Princeton, New Jersey; Merck and Co., Rahway, New Jersey; Chas. Pfizer and Co., Brooklyn, New York.

KSHITISH J. DIVATIA, GRACE GARDNER, JEAN DUFRENOY, and ROBERTSON PRATT

University of California College of Pharmacy, San Francisco, California, September 30, 1950.

Zusammenfassung

Ein gegen Streptomycin empfindlicher Stamm von *Escherichia coli* kann durch tägliches mehrfaches Überimpfen in eine Nährlösung mit steigendem Gehalt an Antibiotikum gegenüber Streptomycin resistent gemacht werden. Dieser Stamm wächst – wenn genügend Zeit gelassen wird – in einer Lösung, die 300 mg Streptomycin pro Liter enthält. Er zeigt auch normales Wachstum in Agar ohne Streptomycin und im gleichen Medium, das 0 bis 100×10^{-8} Mole *p*-Aminosalizylsäure per cm^3 enthält. Die Bakterien wachsen hingegen nicht bei einer Konzentration von 1000×10^{-8} Mole PAS pro cm^3, wenn nicht Streptomycin hinzugefügt wird. In diesem Fall scheint Streptomycin die wachstumshemmende Wirkung großer PAS-Konzentrationen zu neutralisieren.

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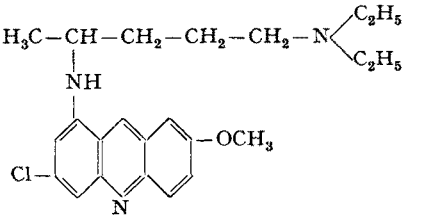
The Colchicine-like Action of an Acridine Derivative

Acridine derivatives have been known as mitotic poisons for many years[1]. In these last years they were investigated chiefly as bacteriostatic and bactericidal substances. Recently we find an important paper of I. LASNITZKI and J. H. WILKINSON[2], who investigated the action of some amino-acridines on the growth of chick fibroblasts: all such acridine derivatives caused disturbance of outgrowth and (or) reduction of mitoses. At the higher concentrations rounding and vacuolisation of nucleoli and vacuolisation of the cytoplasm could be observed. Cells were prevented from entering into division, but there was no interference with the actual process of division, in contrast to colchicine. Therefore abnormal mitoses were not found.

It seemed interesting to study the action on tissue cultures of an acridine derivative that bears a long side-chain and has been already introduced as Atebrin into human therapy, precisely with the aim of preventing the multiplication of an animal cell, the malaria-plasmodium, whose metabolism resembles very closely that of the cells of high vertebrates[3]. It may be important to ascertain whether the long side-chain modifies the cariotropic activity of such compounds in some way.

We used the italian preparation *Italchina* (Farmitalia). Structural formula:



Technique. The cultures were obtained from the cardial region of generally 8–10 days old chick embryos and grown by the hanging-drop technique in a medium consisting of one part chick plasma, one part chick embryo extract, and one part of Tyrode (controls) or Italchina-solution in Tyrode; according to this method (M. I) the acridine derivative acted on the cells during the whole period of growth.

The concentration of the compound definitely acting on growing cells was of course one third of the concentration of the added solution. In some sets of experiments (which are called "M. II" in this paper) we added the Italchina-solution after 24 or more hours of

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Method I Concentration of added Italchina-solution (*c*)	Endconcentration (*c*:3)	Mitotic rate (control culture in parenthesis)		Migration
		after 24 h growth	after 48 h growth	
1:50,000	1:150,000	8·7 (57·2)	20·1 (39·2)	very strongly disturbed
1:100,000	1:300,000	7·2 (42·4)	29·4 (52·2)	very strongly disturbed
1:150,000	1:450,000	7·6 (56·8)	13·4 (38·6)	strongly disturbed
Method II (Italchina added after 24 hours growth)				
1:50,000	<1:50,000	24·4 (59·8)	15·7 (48·1)	very strongly disturbed
1:100,000	<1:100,000	18·1 (59·8)	13·7 (40·1)	
1:150,000	<1:150,000	19·1 (59·8)	14·9 (40·1)	

The prophases seem to be more strongly reduced in number. Higher dilutions of Italchina gave less regular results.

growth: but this second method gave less precise results, the final concentration of the compound acting on cells remaining rather uncertain. All cultures were fixed in Zenker-formol and stained with hæmatoxylin for end examination.

For every experiment six or more cultures (control as well as treated) were used.

Results. While a more extensive study will be published later, we give here a concise account of our observations. The growth of cultures treated according to method I was always impaired: strong disturbance of cell migration, which was very scanty and disordered in comparison to control cultures; large cell vacuolisation; strong decrease of mitotic rate (calculated pro 1000 cells). Treatment according to method II gave slowing down or stopping of growth. Main quantitative findings may be tabulated (see table).

The most significant feature in our experiments is given by the *large number of mitotic changes* (in all tested concentrations), contrasting with the absence of such as remarked by the above authors working with more simple acridine derivatives. We found bridge formation in anaphase, picnotic metaphases, chromosome breaches and detachments, tripolar forms of mitosis, i.e. most of the changes described in connection with colchicine and other antimitotic poisons. Such changes were also found in subcultures on culture medium without Italchina. We may say that our substance shows *in vitro* an action

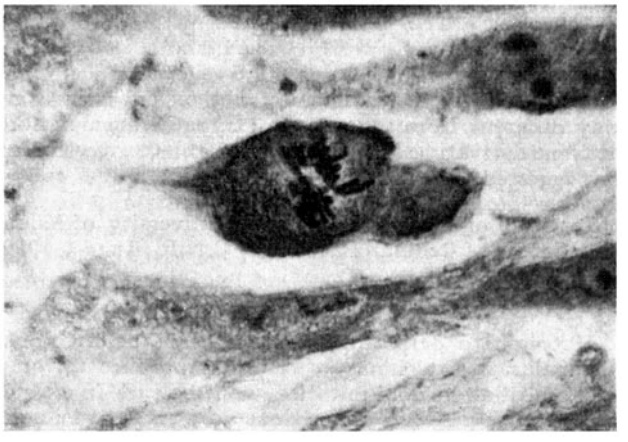

Fig. 2. – Culture as in figure 1. End concentration of Italchina 1:150,000 (M. I.). Disordered metaphase. Enlarged 2500 ×.

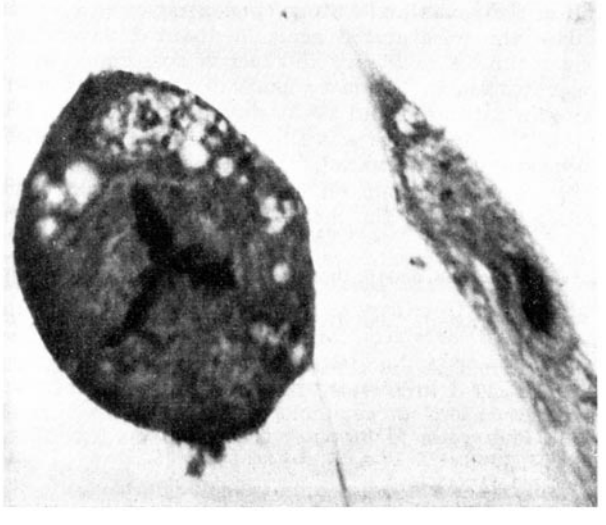

Fig. 1. – Culture of myocardial cells of chick embryo. End concentration of Italchina 1:300,000 (M. I.) Tripolar mitosis. Enlarged 2250 ×.

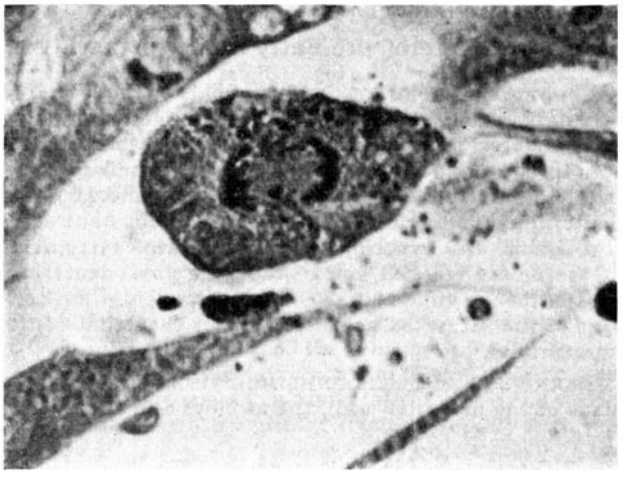

Fig. 3. – Culture as in figures 1 and 2. End concentration of Italchina 1:150,000. Anaphase with detachment of a chromosome fragment. Enlarged 2310 ×.

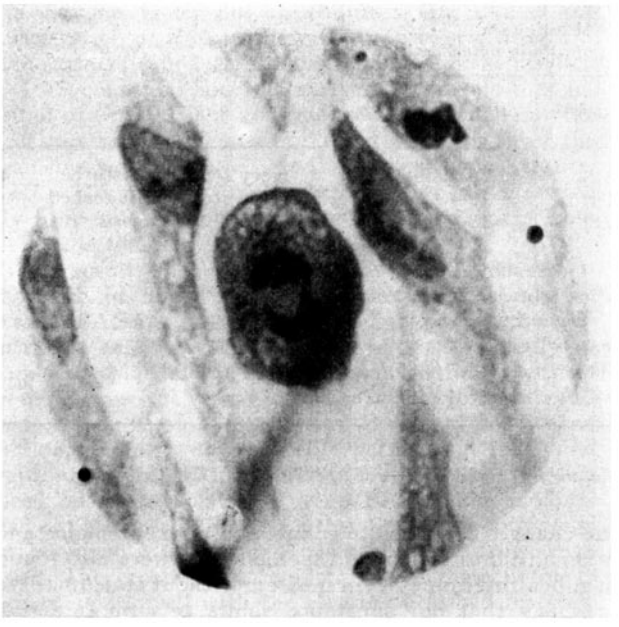

Fig. 4. – Culture of myocardial cells: after 24 h growth a drop of Italchina 1:100,000 added (M. II). Anaphase with bridge formation. Enlarged 1350 ×.

that approaches the colchicine function and is in some way different from the action of trypaflavine and other acridine derivatives. Experiments on tumor growth may be suggested.

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Riassunto

Il ben noto derivato acridinico usato nella terapia antimalarica (Italchina = Atebrina) produce un gran numero di alterazioni mitotiche nelle culture *in vitro* di mioblasti di pollo, in opposizione ad altri derivati acridinici, i quali secondo dati della letteratura impediscono l'inizio della mitosi senza disturbare poi il meccanismo mitotico. Qualche importanza nel modificare l'azione sulle cellule può forse averla la lunga catena laterale del preparato.

Turnover Rate of the Fatty Acids of the Liver

The rate of renewal of the fatty acids of the liver and other organs has been repeatedly determined by making use of deuterium, carbon 13 and carbon 14 as an indicator. In early experiments SCHOENHEIMER and RITTENBERG[1] administered heavy water to mice and kept the deuterium content of body fluids at a constant level throughout the experiment. The saturated fatty acids of the mouse reached half of their maximal deuterium content in the time of 5 to 9 days. The deuterium content of the saturated acids was higher than that of the unsaturated.

BERNHARD and SCHOENHEIMER[2] isolated the fatty acids of the intestinal wall, the kidneys and the liver of the mouse and found the saturated fatty acids to have an appreciably higher deuterium[1] content than had that of the unsaturated ones. They estimate the half life time of the average saturated fatty acid molecule in the liver of the mouse to be about 1 day, while the half life time of the total fatty acids in the rat liver was found by STETTEN and BOXER[2] to be 1·9 days.

The finding of RITTENBERG and BLOCH[3] that the feeding to mice of acetate containing C^{13} in the carboxyl group leads to the formation of fatty acids containing C^{13}, opened the way to the application of C^{13} and C^{14} in the study of the rate of formation of fatty acids. They found a more rapid incorporation of C^{13} in to the saturated than into the total fatty acids. The C^{13} concentration of the carboxyl carbon atoms of the saturated fatty acids was approximately twice as high as the average of all the carbon in the saturated fatty acids. The most plausible distribution which will explain these data is one in which the labelled carbon is present at every other carbon atom; i. e., at the odd number carbon atoms of the fatty acids. Later work[4] showed that at least 25 percent of the carbon atoms of the fatty acids are derived from acetate. Evidence wasalso obtained that acetic acid can furnish every carbon atoms of the molecule.

Recently the rate of turnover of fatty acids has been reinvestigated with the aid of acetic acid labeled by C^{14} in the carboxyl group by Pihl et al.[5]. The percentage renewal of the fatty acid molecules is determined by comparing the C^{14} content of the fatty acid carbon at the end of the experiment with the average value of the C^{14} content of the precursor carbon which prevailed during the experiment. To arrive at the last mentioned data, phenyl-DL-aminobutyric acid was fed simultaneously with labeled acetate to adult rats kept on fat free diet, and consecutive samples of the excreted acetyl derivatives were analyzed[6]. Though the labeled acetate content of the diet was kept constant, the isotope concentration of the acetyl group was found to increase in the course of the 30 days period with about 30% of the initial value. This increase was shown to be due to the catabolism of the labeled higher fatty acids formed during the experiment. The metabolic products of the labeled fatty acids contribute in these long-time experiments significant quantities of labeled acetyl groups to the acetic acid pool which supplies C^{14} to the newly formed fatty acid molecules.

The saturated fatty acids of liver were found to reach half of their maximal isotope concentration in less than 1 day, the unsaturated acids in about 2 days. Much longer time is necessary to reach a corresponding C^{14} concentration in the fatty acids of the carcass, 16–17 days for saturated and 19–20 days for the unsaturated acids. This difference was also shown in recent work of POPJAK and BEECKMANS[7].

In an investigation on the effect of changes in the metabolic rate on the incorporation of C^{14} into tissue

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