the present contacter, does not require exact reference to a simultaneous record of time, indicates instantaneous changes in frequency, and provides a ready-made graph of the (reciprocal) frequency course. Junkmann's[7] original Impulszähler falls into the latter category except for *direct* frequency plotting and for lack of instantaneous information.

As illustrated in Fig. 2, with optimal adjustment, the systolic excursions of the manometer are selectively registered during widely and abruptly varying patterns of respiratory pressure waves, pulse pressure, and mean pressure. The limits of fidelity are those of the mercury manometer, as illustrated by the individual unregistered systole in Fig. 2B (arrow), where a very low pulse oscillation was masked by relatively vigorous respiratory waves during histaminic hypotensive dyspnea.

The principle of automatic compensation to varying hemodynamics, fully present in the contacting instrument of Genuit and Ruppert[6] which inspired the present simplification, was lacking in Fleisch's Pulspelotte and partially present in such manner as to operate against sensitivity in Nyboer's[8] contacter. Electronic cardiotachometers employing the cardio-electric R-wave are, of course, inherently independent of hemodynamics.

It will be apparent that the contacter described is adaptable to many oscillatory phenomena, with provision of a simple engagement for the contacting lever.

Summary. A simple contacting lever, mounted on a carrying lever and engaged with a mercury-manometer cross-wire, selectively registers systolic pulsations. It is used to activate, through a relay, a simple counter-recorder,[1] improved. Individual pulses, tens, and hundreds (etc.) are characterized by amplitude and/or direction of excursion of a single recording lever. Operation and limitations are illustrated.

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Alkaline Phosphatase Activity and Basophilia in Hepatic Cells Following Administration of Butter Yellow to Rats.*

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A cytochemical study[1] has been reported previously of the activity of phosphatase (at pH 5.0) in the cytoplasm of motor neurons during their regeneration. A well-defined increase was found to be associated with the dissolution of cellular basophilic material, chromatolysis, and its regeneration, chromatogenesis.

The alterations in phosphatase activity previously described were in cells which do not divide. It was desirable, therefore, to extend the observations to include regenerating cells in which mitosis can be investigated.

There are presented herewith the results of a study of alkaline phosphatase activity as correlated with basophilia in the hepatic cells of rats fed butter yellow (*p*-dimethylaminoazobenzene) in a diet which renders the liver of the animal susceptible to consequent cancer production.

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A large part of the cytoplasmic basophilic substance is contained in prominent structural entities, called Nissl bodies in the case of the nerve cell. These structures have been shown to consist of a protein matrix with a basophilic component which can be removed by ribonuclease activity.[2,3] They absorb ultraviolet light at 2600 Å,[3,4] and do not react by Feulgen's method. It can be concluded, therefore, that they contain nucleic acid of the ribose type.

Opie[5] described the mobilization of basophilic material in the cytoplasm of liver cells in which degenerative, regenerative, and proliferative changes had been induced by butter yellow. This material was shown by Opie and Lavin[6] to have the characteristics of ribonucleic acid.

The demonstration of alkaline phosphatase in large granules separated from the cytoplasm by Claude[7] and in residual chromosomes isolated from the nucleus by Mirsky and Ris[8] indicates an association of this enzyme, or group of enzymes, with structural and functional components of the cell.

Methods. Thirty-three albino rats (Sherman strain) were offered a diet of unpolished rice supplemented with fresh carrots. Butter yellow, *p*-dimethylaminoazobenzene,[9] was included at a level of 0.06%. Six normal rats of comparable age and weight, offered the same diet but without butter yellow, were studied as controls. At approximately weekly intervals from 7 to 258 days, the animals were killed and studied histologically. The weights at death were from 85 to 380 g.

Thin slices of liver were fixed immediately in ice-cold acetone, imbedded in paraffin, and cut in serial sections. The method of Gomori[10] was employed for the histological demonstration of alkaline phosphatase activity at pH 9.0, temperature 37°C, and period of incubation from 1 to 3 hours. A variety of substrates were used: glycerophosphate, fructose diphosphate, adenylic acid, and ribonucleic acid (from yeast). Adjacent sections were stained by a variety of technics, the most useful of which was that of Giemsa (Wohlbach's modification).[11] In addition, sections were studied for basophilia, when stained with methylene blue and toluidine blue under controlled conditions,[12] before and after incubation in a solution of purified ribonuclease,‡,[13] (enzyme concentration 0.1 mg/cc, pH 6.75, temperature 58°C, duration 2 hours) and desoxyribonuclease,[14] (enzyme concentration 0.1 mg/cc, pH 6.75, Mg ion 0.003 M., temperature 37°C, duration 2 hours).

Results. Although acetone fixation is not ideal, basophilic bodies were prominent in the cytoplasm of the normal rat liver cells stained by Giemsa's procedure. (Fig. 1A). Alkaline phosphatase activity was uniformly slight in both the cytoplasm and the nucleus of these cells (Fig. 1B).

After the administration of butter yellow, however, degeneration with chromatolysis and regeneration with chromatogenesis were present in the cytoplasm essentially as described by Opie.[5]

Early focal chromatolytic changes were demonstrable after 24 days, and were prominent after 45 days, when some cells had lost much or all of their cytoplasmic basophilia and, in some instances, the staining properties of their nuclei as well. After 51 days, basophilia as evidence of chromatogenesis was seen in some cells, although advanced chromatolysis was still predominant. At 95

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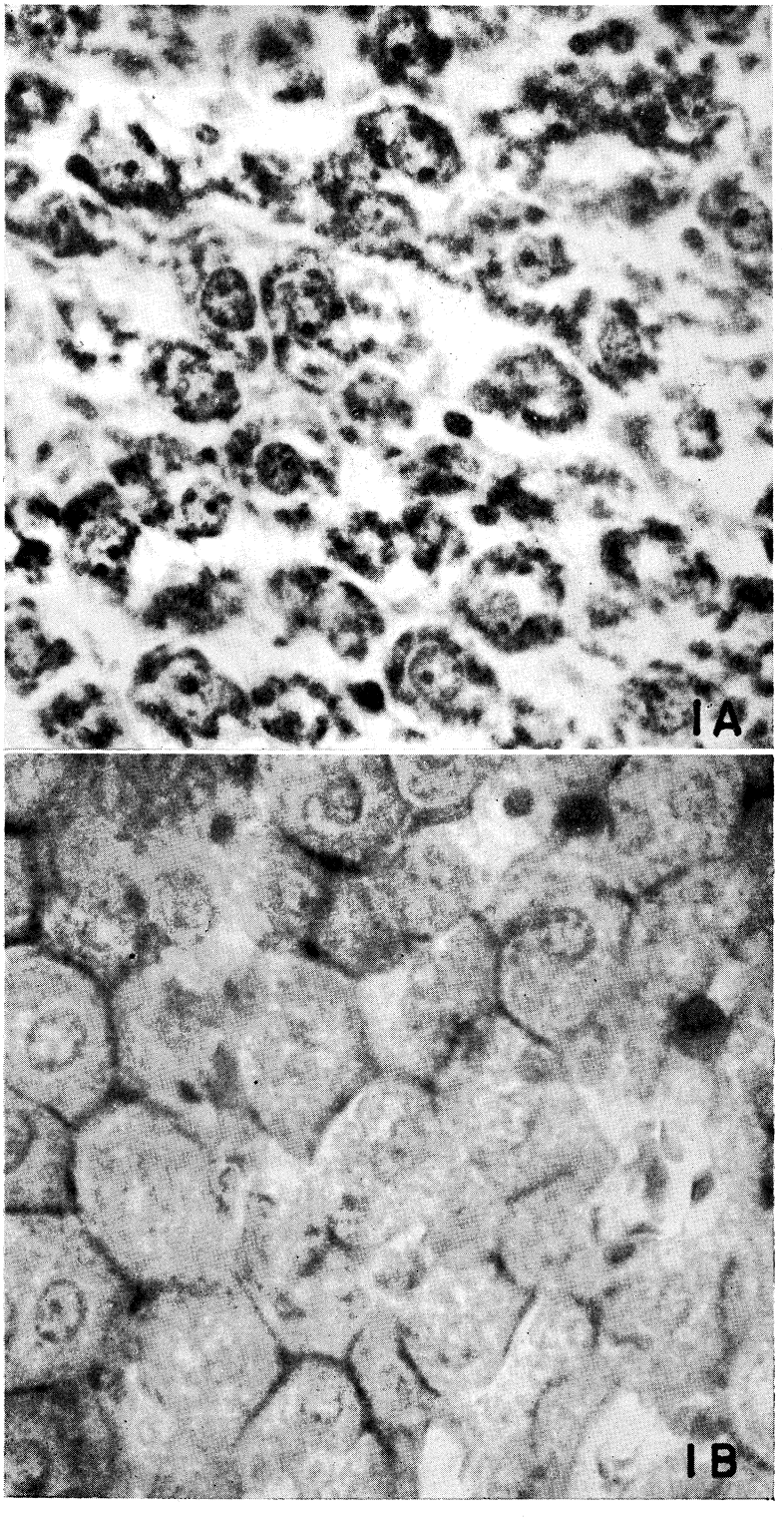

FIG 1A. Normal hepatic cells containing cytoplasmic basophilic material. Acetone fixation. Paraffin section 4 microns. Giemsa (Wolbach's). 500 ×.

FIG. 1B. Normal hepatic cells with slight alkaline phosphatase activity. More conspicuous activity in biliary capillaries. Acetone fixation. Paraffin section 5 microns. Gomori's method. Glycerophosphate substrate, pH 9.0, 3 hours' incubation at 37°C. 500 ×.

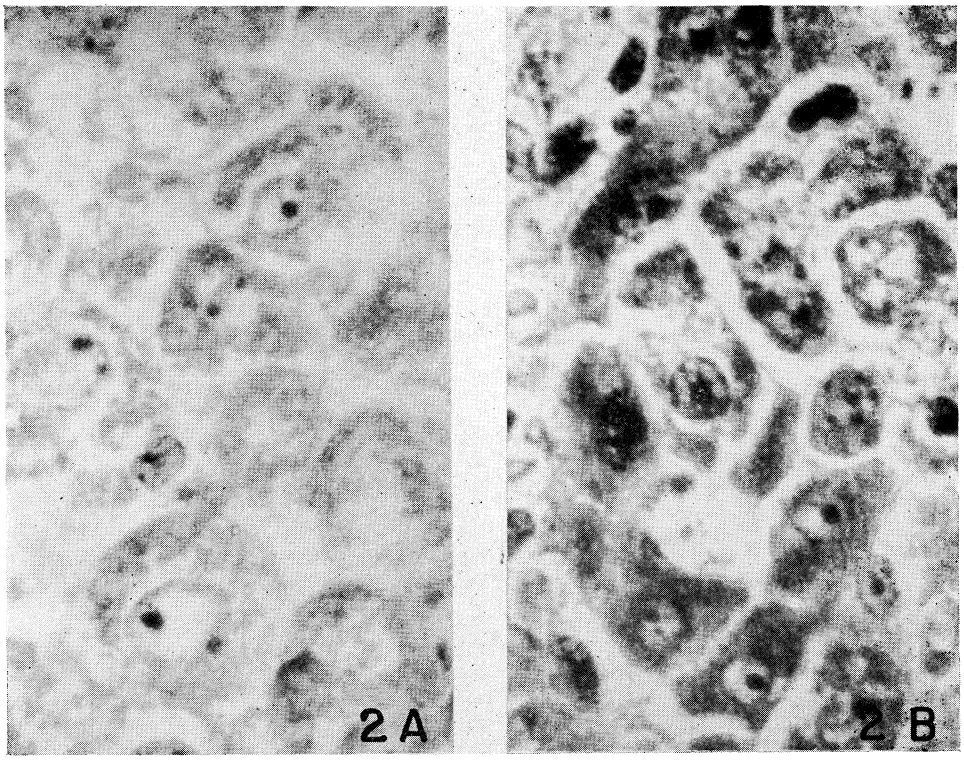

FIG. 2A. Degeneration and chromatolysis in hepatic cells after 95 days of butter yellow administration. Acetone fixation. Paraffin section 4 microns. Giemsa (Wolbach's). 500 ×.
FIG. 2B. Same section as 2A. Focus of regeneration and chromatogenesis in hepatic cells, with prominent cytoplasmic basophilia.

days, cytoplasmic basophilia was intense in foci of regeneration and hyperplasia (Fig. 2B) in sharp contrast to the faint basophilia of cells with persistent evidence of degeneration and necrosis (Fig. 2A). After 197 days, focal basophilic regeneration and hyperplasia were associated with hepatoma, cholangioma, and adenocarcinoma.

A slight tendency toward decreased alkaline phosphatase activity at pH 9.0 was present in association with the hepatic chromatolysis (Fig. 3A). In contrast, an increased activity was frequently present, both in the nucleus (nuclear membrane, chromatin, nucleolus, dividing chromosomes) and to a lesser degree in the cytoplasm in foci of basophilic hyperplastic and proliferating cells (Fig. 3B). This conclusion was established by direct comparisons of sections stained for alkaline phosphatase activity with adjacent sections stained by the Giemsa procedure. In these comparisons, it was clear that, although a good correlation could be drawn between basophilic hyperplasia of parenchymatous cells and a distinct increase in phosphatase activity, the findings were not absolutely uniform.

Observations of cells other than parenchymatous under the conditions of these experiments were of interest. In the epithelium of the bile ducts, no significant alteration in phosphatase activity occurred either during proliferation or actual hyperplasia. Intense, although not necessarily increased, phosphatase activity distinguished mast cells, sometimes present in abundance in the connective tissue around proliferating bile ducts. Conspicuous activity was occasionally present in the sinusoidal endothelium and usually present in the endothelium of the small blood vessels. Moderate activity was in evidence in proliferating fibroblasts in the stroma of cirrhosis and cholangiofibrosis.

In hepatoma, cholangioma, and metastasizing adenocarcinoma, the phosphatase activ-

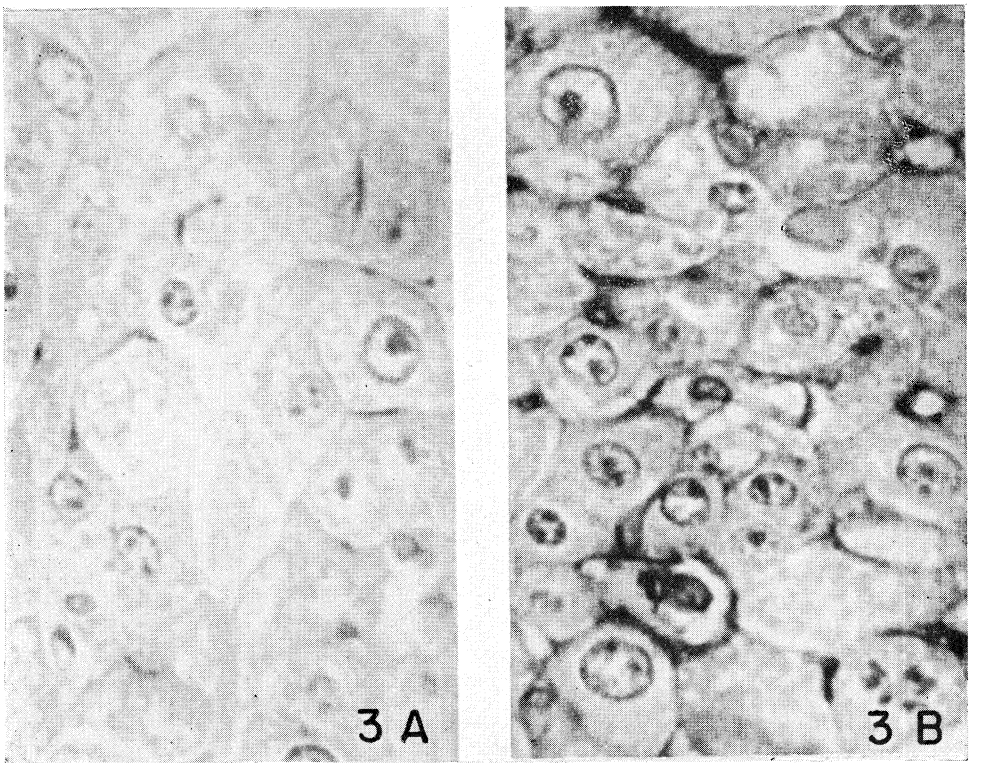

FIG. 3A. Section adjacent to 2A. Same area of degenerating and chromatolytic hepatic cells as shown in 2A, with very little alkaline phosphatase activity. Acetone fixation. Paraffin section 4 microns. Gomori's method. Glycerophosphate substrate, pH 9.0, 3 hours' incubation at 37°C. 500 ×.

FIG. 3B. Same section as 3A. Same focus of regeneration and chromatogenesis as shown in 2B, with increased alkaline phosphatase activity in nucleus (membrane and chromatin), chromosomes, and, to a lesser extent, in cytoplasm of hepatic cells. Activity is conspicuous also in a bile capillary.

ity, variable and focal in distribution, occurred in the tumor cells, particularly in those which appeared to be degenerated or to be associated with necrotic debris.[15]

Summary aand Conclusions. As with the reformation of Nissl substance in the motor neuron, the resynthesis of nucleic acid in the cytoplasm of the regenerating hepatic cell, injured by butter yellow, is accompanied, during certain phases of the reaction at least, by an enhanced activity of alkaline phosphatase. The cellular distribution of the alteration is principally cytoplasmic in the nerve cell, with the optimum pH in the acid range, and nuclear in the hepatic cell, with the optimum pH in the alkaline range.

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