the method of breaking by bringing the tip up against the coverslide (Chambers)[1] frequently results in faulty micro-pipettes, thus causing a considerable loss of time.

The device consists of a frame made of a strip of brass with a thin groove milled out along its center and folded to form a square with the groove inside.

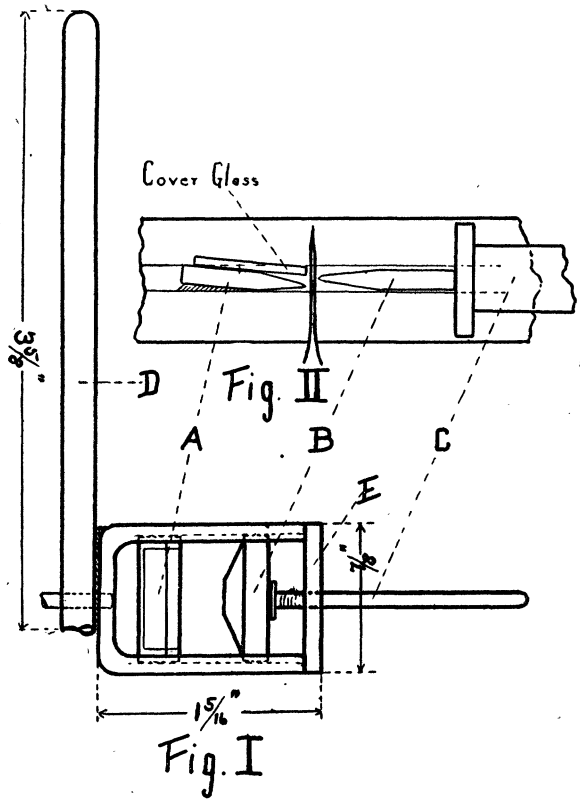

Fig. II

Fig. I

Into this groove are fitted (A) a piece of Gillette safety razor blade and (B) another piece of blade, cut to form a rough wedge front and attached to a holder (C) (in this case an ordinary wire nail) by which it may be drawn back and forth. On top of blade A is a thin piece of cover-glass so placed as to leave a space between it and the blade, just wide enough for the edge of blade B to enter (see Fig. 2). The edges of this cover-glass and blade A which face blade B must be accurately lined up, one above the other. The entire frame is soldered to a rod as indicated.

In operating, the guillotine is supported by the rod in one of the holders of the micrurgical machine under the microscope and the pipette introduced between the blades. The guillotine and the pipette are then approximated to each other until the pipette rests against blade A at the place where the break is to be made. Blade B is then pushed in gently, but firmly against the pipette and the break is made (*cf.* Fig. 2). The blades must be scrupulously clean or the pipette may clog. If greater delicacy in manipulating blade B is desired, the sliding holder to which it is attached may be changed for a screw and a hole may be tapped in cross-bar E to receive it.

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SPECIAL ARTICLES

RESULTS SECURED BY APPLYING THE FEULGEN REACTION TO FIBROBLASTS AND SARCOMATOUS CELLS IN TISSUE CULTURES

WHEN a cell once becomes cancerous the change in it is inherited and transmitted to all its descendants within the afflicted person. This persistence of malignancy has led investigators to suspect that the nucleus is profoundly modified; but no constant microscopically visible deviation from the normal has yet been demonstrated. With the idea of again attacking this problem Dr. Alexis Carrel very kindly gave me a series of tissue cultures of normal fibroblasts and of sarcomata and to them I have applied the reaction recently introduced by Feulgen[1] for the demonstration of thymonucleic acid in the hope that thereby some slight difference associated with malignancy might be brought to light.

The reaction depends upon the formation of a relatively insoluble dark purple compound in regions of the nucleus containing thymonucleic acid. The distribution of the reacting material is very restricted. It does not occur, for example, in either the Nissl bodies of nerve cells or the chromidial substance of gland cells, although both of these materials stain like nuclear chromatin with basic dyes, and like it also contain detectable amounts of masked iron. The exact technique employed is described elsewhere.[2]

Special precautions were observed in order to make the comparison between the normal and malignant cells as close as possible. In the cultures the normal fibroblasts and sarcomatous cells were planted side by side in the same flasks. After their rate of growth had been determined they were fixed simultaneously by flooding the flask with a mixture consisting of equal parts of absolute alcohol and saturated aqueous corrosive sublimate. The layer of coagulated plasma covering the bottom of the flask and containing both groups of cells was then removed and its edges cut

[1] Krause, R., *Enzyk. f. mikr. tech.*, 1927, Vol. 3, pp. 1729–1732.

[2] Cowdry, E. V., SCIENCE, 1928.

so that a longer stretch of the layer of plasma remained in continuity with the fibroblasts than on the other side of the adjacent sarcomatous cells. This was done for purposes of identification. After dehydration and embedding, the block was oriented in such a way that corresponding serial sections of fibroblasts and sarcomatous cells were cut simultaneously. Since they were mounted in parallel rows (attached together by the coagulated plasma) on the same slides, both were subjected to exactly the same microchemical treatment. The experiments were all made at the Rockefeller Institute for Medical Research in New York.

In Experiments 1 and 2 normal rat fibroblasts and the cells of rat sarcoma X (from the Crocker Foundation) were cultivated close together in the same flasks. In both experiments the sarcomatous nuclei gave a much stronger Feulgen reaction than did those of the fibroblasts. But it was thought that the difference in the nuclei might be in a sense accidental or that the reaction given by the nuclei of fibroblasts might normally be somewhat inconstant and subject to variation, in which event no significance could be attached to the difference observed.

Accordingly, Experiment 3 was planned to determine the influence upon the reaction, if any, of differences in nutrition. Cultures of Carrel's old strain of chicken fibroblasts were made in two flasks in tyrode and embryonic extract, respectively. Simultaneous fixation was secured by immersing both flasks in a beaker containing a generous supply of the alcoholic sublimate mixture, after which the groups of cells were removed and carried through all steps in the technique together. Examination showed that the reaction of the nuclei was apparently identical in both, being faint like that of the rat fibroblasts, and this despite the fact that the cells cultured in tyrode had been starved as compared with the others living in embryonic extract.

Experiment 4 was then made as a check on Experiments 1 and 2. The technique was the same except that as a further control the normal fibroblasts were planted between two fragments of sarcoma. Again the results were equally striking. As one passed from the first sarcoma to the normal the purple color of the nuclei faded abruptly only to suddenly increase again on reaching the second piece of sarcoma.

The object of Experiments 5 and 6 was to determine whether the nuclei of another sarcoma exhibited an equally strong Feulgen reaction. Cultures were accordingly made in duplicate in diluted liver digest of rat sarcoma X and of the Jensen rat sarcoma placed side by side. With both tumors the reaction was found to be intense and indistinguishable in degree. Evidently, therefore, the intense reaction is just as characteristic of the two sarcomata as the feeble reaction is of the fibroblasts of the rat and of the chick in the cultures investigated.

In order again to test the difference noted in Experiments 1, 2 and 4 between the reaction of the nuclei of normal rat fibroblasts and those of rat sarcomata four more experiments were performed. In the first two (Nos. 7 and 8) normal rat fibroblasts and rat sarcoma X were studied as cultivated in diluted liver digest. In both of them the sarcomatous cells gave regularly and constantly a more intense Feulgen reaction than the normal fibroblasts. In the second two (Nos. 9 and 10) a comparison of normal rat fibroblasts and the Jensen rat sarcoma cells was made under similar conditions and gave the same results.

The conclusion then seemed inevitable that, under the conditions named (and this is a necessary qualification), the nuclei of both tumors are much richer in the substance giving the Feulgen reaction, which is probably thymonucleic acid, than the normal rat fibroblasts. This result was rather unexpected because no such distinction was observed by either Roskin[3] or Ludford[4] both of whom studied fragments of excised tumors by Feulgen's method.

To discover whether the same difference also holds in neoplastic cells *in vivo*, as was found in the tissue cultures, Dr. J. B. Murphy very kindly gave me a number of mice with tumors for examination. These included one sarcoma (180), several spontaneous mammary cancers and a transplanted mammary cancer (Bashford, 63). With each a variety of other tissues were fixed in the same fluid and sections were mounted on the same slide so that the microchemical reaction was applied alike to the tumors and normal tissues.

It was found that the thymus gland always gave a more pronounced Feulgen reaction than the tumors, but this was expected because the thymus is known to contain unusually large amounts of thymonucleic acid. In the other fragments of tissue the nuclei of the tumor cells were strongly stained so that the extent of the neoplasms could be readily seen by naked-eye inspection of the slides. Although this was the case in a gross way, when it came to a direct microscopic comparison between the nuclei of individual sarcomatous cells and those of unaffected fibroblasts no noticeable difference could be made out; yet it is possible that the introduction of accurate methods of estimating the color values might reveal a slight divergence between them. Just how the nuclei of the

[3] Roskin, G., *Zeit. f. Krebsforsch.*, 1925, Vol. 22, pp. 472–479.

[4] Ludford, R. J., *Proc. Roy. Soc.*, Series B, 1928, Vol. 102, pp. 397–405.

mammary cancer compare with those of normal mammary glands remains to be determined.

It is unsafe at present to try to explain why such an outspoken difference was noted between the nuclei of sarcomatous cells and those of normal fibroblasts in the tissue cultures examined, while a similar difference could not be detected in pieces of excised tumors. The results are merely reported as they stand for the present without interpretation.

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THE PLEISTOCENE ELEPHANTS OF SANTA ROSA ISLAND, CALIFORNIA[1]

W. G. BLUNT'S discovery of fossil teeth of an elephant on Santa Rosa Island, one of the Channel Islands off the coast of southern California, was recorded by Stearns[2] in 1873. Since that time this interesting and significant occurrence has been referred to by several authors. Hay[3] has recently summarized the available information and recognizes the presence of *Elephas imperator* and of an undetermined species.

During the past year Dr. Spencer Atkinson and Mr. J. A. Barbieri, of Pasadena, secured a fragmentary elephant skull on Santa Rosa Island and presented the specimen to the California Institute of Technology. Through the courtesy of the Vail Company of Los Angeles, owners of the island, the California Institute, with the cooperation of the Carnegie Institution of Washington, have been given the opportunity to investigate the occurrence, and facilities were kindly made available to collect further remains.

Santa Rosa Island is the second largest of four islands separated from the mainland by the Santa Barbara Channel, which has an average width of twenty miles and an average depth of approximately six hundred feet. The geology of this insular area has been recently studied by Kew.[4] The Pleistocene deposits in which the elephant remains were found occur in the northwestern portion of the island, where they are well exposed in the sea-cliffs and are eroded into bad lands along the stream courses or canadas. These deposits are largely of terrestrial origin and consist of clays, sands and gravels, reaching a thickness in places of more than seventy-five feet. At one locality a whale vertebra was found at the base of the Pleistocene strata. The beds rest with marked unconformity upon northward-dipping, marine Miocene sandstones and shales and have suffered little or no deformation. Since their accumulation, however, the Pleistocene beds have been subjected to subsidence, planation and uplift, for at least one well-defined terrace cut into the deposits is now situated at an elevation of seventy-five feet above sea-level. On this terraced surface occur sand dunes and shell mounds in which Indian burial sites are frequently encountered.

The collections secured by the California Institute include a number of teeth, parts of skulls and skeletal material. Occasionally several skeletal elements and teeth are found associated in the deposits. Usually the remains are scattered. One curious feature of the occurrence is the apparent total absence of associated mammalian types. The proboscidean remains are referable to the genus *Elephas*. The individuals exhibit considerable variation in size, and this is undoubtedly to be ascribed in part to differences in age. A survey of the collection as a whole yields the impression rather strongly that the elephant types were of relatively small size. Some of the forms may have a height of six to eight feet as measured at the shoulder. The larger individuals are perhaps comparable in size to the American mastodon and are certainly smaller, possibly considerably smaller, than the Pleistocene mammoths of the southwestern United States.

While the Santa Rosa Island elephant has been determined as representing the species *Elephas primigenius* Blumenbach and *E.* (*Archidiskodon*) *imperator* Leidy, the difference in size, coupled with differences noted in the skull and dentition, seem quite clearly to distinguish the island form as a distinct species for which the name *Elephas exilis* is here proposed. The type of the species is No. 14 Calif. Inst. Tech. Coll. Vert. Pale., a skull and mandible including four cheek-teeth and two tusks. A fuller description of the species will be published elsewhere.

The relatively small size of the Pleistocene elephants of this region suggests the interesting possibility of a dwarfing of these forms induced by insular isolation. This brings to mind the occurrence of the pigmy elephant *Elephas melitensis* Falconer in the bone caverns of Malta and of the related *E. mnaidriensis* Adams of Sicily, types which have become classic in paleontological literature as examples of a dwarfing of large mammals resulting from island isolation. While the factor of isolation may have been influential in producing the characters exhibited by the Santa Rosa Island elephant, it should be clearly recognized

[1] Paper read in part at the meeting of the Cordilleran section, Geological Society of America, Berkeley, California, March 3, 1928.

[2] Stearns, R. E. C., Proc. Calif. Acad. Sci., Vol. 5, p. 152, 1873.

[3] Hay, O. P., Carnegie Inst. Wash. Pub. 322B, pp. 42, 43 and 51, 1927.

[4] Kew, W. S. W., Bull. Geol. Soc. Amer., Vol. 38, pp. 645–654, 1927.