Abstracts of Papers Presented at the Fifth Annual Meeting: The American Society for Cell Biology

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Abstracts of Papers Presented at the Fifth Annual Meeting

THE AMERICAN SOCIETY FOR CELL BIOLOGY

THE BENJAMIN FRANKLIN HOTEL
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vinylpyrrolidone or crystalline bovine plasma albumin. However, attachment and extensive outgrowth occurred with fetal calf serum, fraction IV-4 of bovine serum, or fetuin. Fetal calf serum after exhaustive dialysis, and at a concentration of only 1 per cent, still supported extensive outgrowth.

Attempts are continuing to define the macro- and micromolecular requirements of this system and to use it for studying the effect of viruses on early development.

Supported by USPHS Research Career Program award 6-K3-HD-21,269-01A1, NSF grant GB-617, and American Cancer Society institutional grant IN-38-G.

269 A Comparison of the Antilethal and Antileukemic Activities of Sheep Spleen Extracts (Radiation-Leukemia-Protection Factor)

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The efficacy of sheep spleen extracts in preventing the appearance of lymphosarcoma after exposure to x-rays of C57BL mice was reported by Berenblum *et al.* (*Blood*, 1965, **26**, 8). These experiments have been repeated, and the activity of 40 per cent (*w/v*) and 10 per cent extracts demonstrated. In one experiment with 200 mice, the corrected incidences of lymphosarcoma after 401 days in control and treated males were 0.739 ± 0.049 *versus* 0.308 ± 0.035, and in females 0.735 ± 0.036 *versus* 0.263 ± 0.025. There was no significant interaction, and the results for both sexes were pooled for testing the significance of the difference between all treated and control animals. The difference was significant at the 1.8 per cent level. Two other experiments yielded similar results, and for a fourth group, treated with a 10 per cent extract, $P = 0.073$.

To test the antilethal action of 40 per cent extracts, groups of 20 to 50 mice received extracts, or saline solution, before or after exposure to single doses of x-rays ranging from 550 to 800 r. Mice of the C57BL, C3H, or Swiss strain were used in individual experiments. The day of death (to day 30) was recorded. The results of these experiments, unlike those of the experiments with antileukemia groups, were extremely variable, and in no case could we consistently demonstrate either a protective or an adverse effect of the extracts. These experiments indicate that the radiation-leukemia-protection factor (RLP), which prevents lymphosarcoma after exposure to fractional doses of x-rays (188 r per week for 4 weeks), does not protect mice from death after exposure to single large doses of x-irradiation.

Supported by AEC contract AT(11-1)1391 and the Indiana Elks.

270 A Formaldehyde-Glutaraldehyde Fixative of High Osmolality for Use in Electron Microscopy

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A combined formaldehyde-glutaraldehyde fixative has yielded excellent fixation of a wide variety of tissues. 2 gm of *para*formaldehyde powder are dissolved in 25 ml water by heating to 60–70°C and stirring. One to three drops of 1 N NaOH are added with stirring until the solution clears. A slight milkiness may persist. The solution is cooled, 5 ml of 50 per cent glutaraldehyde (Biological Grade, Union Carbide Company) are added, and the volume is made to 50 ml with 0.2 M cacodylate or phosphate buffer, pH 7.4–7.6. The final pH is 7.2. If cacodylate is used, 25 mg $CaCl_2$ anhydrous is added. Slabs of tissue 3 to 4 mm thick are fixed at room temperature for 20 to 30 minutes and are then diced into small blocks, and fixation is continued at room temperature for 2 to 5 hours. The blocks are washed for 3 to 12 hours in cold 0.1 M buffer, are postfixed in cold 1.33 per cent osmium tetroxide buffered with *s*-collidine for 2 hours, and are embedded as usual.

It is of interest that the osmolality of this fixative is about 2010 milliosmols per kg. Despite this high osmolality, shrinkage is unusual except when the fixative is perfused or applied to free-

floating cells and monolayers; then dilution seems desirable (Williams and Gould, work in progress). Tissue fluid in slabs of tissue probably dilutes the fixative as it penetrates, reducing the osmolality somewhat. Myelin figures are less commonly seen than with formaldehyde or glutaraldehyde fixation alone, and all cell components are well preserved except lipid droplets, which are extracted. Microtubules are particularly well preserved. It is surmised that the formaldehyde penetrates faster than the glutaraldehyde and temporarily stabilizes structures which are subsequently more permanently stabilized by glutaraldehyde.

Supported by grant HE-09125 from the NIH, USPHS. The author is the recipient of a Lederle Medical Faculty award.

271 Electron Microscope Studies on the Oocyte of a Holothuroid Echinoderm, *Thyone briareus*

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Electron microscope studies of glutaraldehyde–osmium tetroxide–fixed oocytes of the holothuroid echinoderm *Thyone briareus* reveal from one to many stacked annulate lamellae in the ooplasm adjacent to the nuclear envelope. These structures also occur in other regions of the ooplasm. At times a fibrillar or granular network extends between the annuli of adjacent lamellae. Continuity of annulate lamellae with lamellar and vesicular forms of the endoplasmic reticulum is commonly observed. Several ooplasmic regions contain single rows of lamellar endoplasmic reticulum alternating with single rows of mitochondria. In some of the rows, granule-covered vesicles appear to be in process of forming the lamellae of endoplasmic reticulum, which in larger oocytes may be organized into long stacks without any preferential association with mitochondria.

The Golgi complexes are prominent and widely distributed especially in the peripheral ooplasm. The presence of a granular matrix within many of the Golgi vesicles which subsequently increase in size suggests that at least one component of the yolk is formed in association with the Golgi complex. Striated, fibrillar elements and microtubules are sparsely distributed throughout the ooplasm. The apparent fusion of large granules of low density with the plasma membrane suggests a possible mechanism for the formation of the vitelline membrane. Occasionally, cilia are present in certain of the follicle cells, in which case a Golgi complex and microtubules are associated with the basal body.

Supported by grants HD-00699 and GM-09229 from the NIH, USPHS.

272 Effects of Calf Thymus Histone on Embryonic Cells and Tissues

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Histones are suspect as regulators of genetic activity. Goodwin and Sizer report (*Science*, 1965, **148**, 242) the effect of calf thymus histone (CTH) in regulating lactic dehydrogenase activity from 14-day chick embryo brain tissue. To demonstrate true regulatory activity, cytotoxic effects should not be encountered. However, such effects are hereby shown to be severe and immediate.

In three separate experiments, results of which were identical, whole chick embryos 8 and 14 days of age, and 14-day embryo brain alone, were minced and prepared as cell suspensions. The suspensions were incubated for periods up to 1.5 hours in Tyrode solutions containing varying amounts of CTH.

By eosin dye exclusion tests, at timed intervals, the LD_{50} was determined at 25 μg/ml. At 50 μg/ml, all cells were dead. The lethal effect occurred at zero time, and did not increase with time of incubation. In contrast, diazotized CTH revealed no toxicity at 500 μg/ml.

If CTH is added to an extract medium, a precipitate is immediately formed. Therefore, in separate experiments, 7-day chick embryo back skin was incubated for 1 hour prior to culture in Tyrode solution containing CTH in concentrations