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L.E.O.
P^{32} experiments on leukemic patients - P. 7
P^{32}, Na^{24}, thyroglobulin + I^{131} tagged iodofluorescein experiments on cancer patients - P. 4

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LABORATORY OF EXPERIMENTAL ONCOLOGY

SEMI-ANNUAL REPORT
December, 1948

Reference is made to the Annual Report 1947-48 from the Laboratory. It is possible to report that steady progress is being achieved toward the full development of the program, and in several fields of investigation.

ADMINISTRATIVE

The research ward of the Laboratory was opened in July, 1948. In September, the isotope unit (a collaborative project with the Division of Radiology and Radiation Laboratory) was initiated. In November, the pathology unit (a collaborative project with the Division of Pathology) started operations.

The total personnel of the Laboratory, as of December 1, and not including the pathology unit, numbered 47, of which 10 were scientists, 13 technical or administrative assistants, 9 nurses, 1 dietitian, 2 resident physicians, and 12 ward helpers, janitors, etc. Of these, 17 are employed directly by the National Cancer Institute, 12 are employed on the grant-in-aid from the National Cancer Institute, 5 are employed by the University on other funds, and 2 are research fellows (National Cancer Institute and Atomic Energy Commission).

The budget of the Laboratory remains essentially as indicated in the Annual Report.

Most of the key equipment has been received and is in active operation. Mr. Jack Theler has kindly donated a passenger vehicle to the National Cancer Institute for use by the Laboratory.

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The research ward, up to December 1, has had 47 new patients, which occupied the ward for a total of over 1,200 days. This represents an average daily census of approximately 10 patients, and, with readmissions, an average hospitalization of 25 days per patient. There have been 14 deaths and 14 autopsies.

RESEARCH PROGRAM

I. Physiology.

A. Clinical.

1. A device for measuring physiological pressure phenomena using the bonded electrical wire resistant strain gage has appeared in the October 1948 issue of the Review of Scientific Instruments. The report on the device for measuring intraarterial and intracardiac blood pressures is in preparation. The device is currently being used in a study on intra-arterial pressures in hyperadrenalism states.

By a constant infusion of epinephrine it is possible to create a clinical state similar to one in pheochromocytoma. By giving adrenalytic drugs such as benzodioxane, DHO-180 and DHE-45, the effect of these drugs upon the hyperadrenal state can be observed. This study was undertaken to obtain information on the vasomotor responses and vasopressor mechanisms in patients with neoplastic diseases, combined with a study of the effectiveness of adrenalytic drugs as a test for pheochromocytoma. Continuous blood pressures are recorded by the cuff and by intra-arterial methods; simultaneously, multiple cardiographic leads, respiration and pulse tracings are taken. Ten patients have been studied to date.

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2. The method for determining pulmonary circulation times by means of the oximeter have been completed and the report has been submitted. It is now possible to determine total pulmonary circulation time, right heart and left heart circulation times. There is direct, constant relationship between anoxia and left heart circulation time, so that with increasing grades of anoxia or decreasing arterial oxygen saturation there is a shortened circulation time. By correcting for body surface area an index of approximately 3 to 3.5 seconds left heart time is obtained for normal subjects. A preliminary impression from our data is that patients with neoplastic diseases have considerably longer circulation times per square meter body surface area. The slope of the circulation time curve can be shifted by hemoglobin, red blood cell levels, and various vasoconstricting drugs. A study of the curve at 6, 18, 24, and 48 hours after complete digitalization by cedalanid reveals a marked change in the slope, which may be related to the vasomotor tone of the pulmonary vessels. It is hoped to adapt this method for studying the vasomotor tone of the pulmonary capillary bed of patients with neoplastic diseases, especially those with pulmonary carcinoma or pulmonary metastases.

3. A study is being carried out on the effect of introducing large numbers of white cells into compatible recipients and following the disappearance of these transfused white cells. Confirming the original data by Murphy and Isaacs in 1925, there is a prompt removal of large numbers of white cells from the peripheral blood, and this disappearance takes place within 5 minutes. One patient developed a reaction to the rapidly infused cells. There was a decrease in total white cell count from the initial count of 18,000 to 3,000, despite the fact that the patient

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was transfused with 530 cc of blood containing 150,000 white cells per cubic millimeter. Apparently the mechanism for controlling the level of white cells in the blood cells in the blood is rapid and much efficient. There is some indication that the lungs play an important role in this mechanism.

4. Studies on two patients with open gastrostomies have revealed that there are gastric potentials within the stomach which can be measured by a wick electrode and a D-C amplifier. Potentials are not affected by anion or cation secretion of the stomach and appear to be steady. Difficulty in maintaining a gastric electrode in the specific area of the stomach of a normal subject is presently delaying the advance of this project. Use of double catheters through the stomach tube is the most possible solution being considered at present.

5. The isotope unit of Drs. Low-Beer, Scott and Lamson has carried out the following studies on the deposition of radioactive materials in patients: P^{32} (8 studies), Na^{24} (2 studies), thyroglobulin with I^{131} (2 studies), di-iodo-fluoroscein with I^{131} (1 study). A study on a patient with an open gastrostomy, open salivary fistula and a jejunostomy showed that Na^{24} given intravenously appeared in all 3 secretions within 3 minutes. Sodium appearance curves were obtained for continuous gastric secretion and following histamine stimulation. Demise of this patient halted the study.

B. Laboratory.

1. Electric potential studies on growing chick embryo have proceeded slowly. By borrowing a suitable photoelectric pen recorder from the Shell Oil Development, it is now possible to record the egg potentials

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continuously for the entire 21-22 day life-cycle of a chick embryo. In general, the continuous recordings confirm the discontinuous measurements which were obtained previously, and to date approximately 46 eggs have been studied. Potentials develop markedly after the tenth day and generally have a positive polarity, reaching the extreme of 20 to 50 millivolts between the fourteenth and eighteenth day. During the differentiation period of the first five days there is no potential beyond ± 3 millivolts. It has been observed that lowering of temperature causes the potential to move toward the positive pole.

2. A method for taking electrocardiograms in mice has been developed. Serial electrocardiograms of mice transplanted with a hepatoma and a lymphoma have shown no abnormal repeatable pattern in the electrocardiogram at any time.

Study of the effect of stilbamidine on mouse electrocardiograms has shown that stilbamidine in small doses causes permanent heart block and respiratory paralysis. Patterns obtained during the development of heart block in the normal mouse cardiogram have been quite spectacular. The collected data are presently being analyzed.

3. A study of formed elements of the blood exposed to ultrasonics frequencies of 635,000 times a second has advanced to the determination of 32 samples of blood on 30 normal subjects. The destruction of the white blood cells falls logarithmically with a range of approximately 0.9 to 6.7 minutes for destruction of 50 percent of the white cells. Hemogram differentials by supravital staining on the same samples have shown that the lymphocytes are less resistant to ultrasonics vibrations than polymorphonuclears. Red cell destruction by the same method has shown a half-life of the red

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cells of approximately 4 minutes, with a range from 1 to 5.5 minutes. The report on this work is in preparation.

Preliminary studies on leukemic cells have shown them, surprisingly, to have a marked ability to withstand the ultrasonics vibrations for half-lives of approximately 3 times that of the normal cells.

II. Chemotherapy.

A. Clinical.

1. A report has been submitted on the first 27 cases treated with HN2 before July 1, 1948. Seventeen additional cases have been treated on the research ward, and an equal number in other hospitals of the area. The standard procedure now employed is 0.3 mg. per Kg. body weight in a single dose, with repetition of up to 0.6 mg. per Kg. if the smaller dose produces incomplete effects and if the hematologic status of the patient allows the therapy some 4 to 6 weeks later. An alternate series of administration of intravenous procaine with HN2 to allay nausea and vomiting has been started; the evidence so far has been equivocal.

2. A series of patients treated with diopterin or teropterin has been discontinued after 17 cases, 8 of whom received HN2 in addition. In no case was there objective evidence of any effect on the progress of the neoplastic disease; occasional temporary feeling of well-being, as reported by Farber and Lehr, has been observed but attributed to psychogenic effects.

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3. Six patients with acute leukemia have been treated with 4-aminopteroyl-glutamic acid (aminopterin). Four of these patients died within 2 weeks while still on therapy; 2 patients remain alive after approximately 4 weeks of treatment. There has been no startling improvement, although the progress of the disease apparently abated in 2 of these patients with regression of lymph nodes, spleen and gum lesions.

4. Three patients with leukemia have been treated with P^{32} by the isotope unit, and one patient with lymphosarcoma received x-ray therapy.

5. A preparation which has received considerable interest locally, crystalline chymotrypsin, is being tried in a series of patients with a variety of neoplastic diseases. So far, 10 patients (8 on the research ward and 2 in other hospitals) have received or are receiving daily injections of 20 to 60 mgm. of the preparation, being furnished by the Kuster Laboratory of San Francisco. In none has there been any evidence of effect upon tumor growth or clinical condition, and 4 patients have died.

6. Three patients with melanoma received oral monobenzyl ether of hydroquinone, a chemical known to block melanin formation. In all three there was apparent increase in the rate of tumor growth after being on the chemical for about 3 weeks. Histologic section of the tumors on two patient, however, showed only increased vascularity of the tumors.

7. A collaborative study with Hooper Foundation of the Medical School has been undertaken to study the effect of virus infections in patients with neoplastic disease. Two viruses, the cat panocytopenia virus (a non-human pathogen) and lymphopathia venereum, were chosen for the first studies. The studies are based on laboratory reports that viruses grow

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selectively on carcinoma tissue of mice, and at least one has been reported to have inhibiting properties on such tumor growth. A direct application to man was deemed desirable, since the high species specificity of viruses makes results of animal studies inapplicable to man.

B. Pharmacology.

1. Studies of the effect of stilbamidine upon the human heart have confirmed previous observations that there is a ST-wave depression and a T-wave change limited usually to the left ventricle precordial leads with therapeutic doses of stilbamidine. The analysis of the data is nearing completion and needs the attention of clinicians using this drug.

III. Biochemistry.

1. Study of the effect of glucose administration on gastric lactic acid production in patients with gastric carcinoma, and in controls without gastric carcinoma have been continued.

To date, 18 determinations have been carried out, 5 gastric cancer cases and 13 non-gastric cancer controls. The results appear to indicate that there is generally a rise in gastric lactic acid following administration of glucose to patients with gastric carcinoma; whereas in non-carcinoma cases no significant change in gastric lactic acid is observed. In two cases of gastric ulcer where a rise in gastric lactic acid was observed following glucose, there was a marked and concomitant rise in gastric pH, indicating a probable regurgitation of intestinal contents.

The conclusion is reached that the gastric lactic acid present in gastric carcinoma is only partially the product of bacterial fermentation. Some lactic acid is also due to the glycolytic activity of the tumor. Since

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in some instances there is an distinct rise in the gastric lactic acid following glucose administration to patients with gastric cancer, the procedure used cannot be applied to the detection of gastric carcinoma.

2. Using oxidation of catechol as a measure of polyphenoloxidase activity, it was found that enzyme activity varied directly with the concentration of plasma. Oxidation of catechol in the presence of leukemic plasma increased, apparently logarithmically, as the temperature was raised from 25° to 43°, while the pH was kept constant at 7.4, or as the pH was raised from 6.6 to 9.2, with the temperature kept constant at 37°. Cyanide completely abolished oxidation of catechol in the presence of leukemic plasma. Even the auto-oxidation of catechol was inhibited under these circumstances. Cyanide, in the absence of leukemic plasma, has no effect on the auto-oxidation of catechol.

Using the conditions of pH 7.4, temperature of 37°, and 1 cc. of serum, "neutralized" to remove bicarbonate, polyphenoloxidase activity was demonstrated in the serum of one patient with myelogenous leukemia and one with carcinoma of the larynx. No carefully controlled study has as yet been carried out with normal blood. There are indications, however, based on two determinations with normal blood, that the polyphenoloxidase activity in normal blood is kept in check by an inhibitor, such that oxidation of catechol in the presence of normal serum is inhibited for considerable lengths of time.

3. One patient with lymphatic leukemia was placed on a metabolic balance study and the nitrogen, sodium and potassium balance determined throughout a period of over 2 months, during which time the patient received .3 mg. of HN2 followed after 3 weeks with .6 mg. of

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HN2 per Kg. body weight. Immediately following the .6 mg. dose of nitrogen mustard, the patient promptly went into a marked negative nitrogen balance and excreted large amounts of sodium and potassium. The significance of the findings is as yet not clear, and it is the intention to repeat the study on 3 patients under better controlled conditions. A possible explanation of the potassium excretion following nitrogen mustard therapy may be in the breakdown of lymphoid tissue which is high in potassium.

4. The study on blood histamine in myelogenous leukemia is continuing, in collaboration with the Permanente Hospital. Another patient with chronic myelogenous leukemia showed a marked rise in blood histamine following epinephrine. An attempt is being made to isolate histamine chemically from the blood of this patient. Histamine values of the blood following adrenalytic drugs is being studied, and preliminary data suggest that these drugs markedly decrease the histamine values.

5. Laboratory chemotherapy.

a. Studies on the possible chemotherapeutic effect of 2,4-D on lymphoma-bearing mice have been extended to several esters of the compound. These are: (1) methyl ester, (2) isopropyl ester, (3) amyl ester, and (4) "lorol" ester, all DuPont products.

A preliminary investigation indicated that the toxicities of the esters were of the same order of magnitude as that of 2,4-D. Strain A mice weighing approximately 25 gm. tolerate daily doses of 2-3 mg. of 2,4-D, or equivalent amounts of the esters.

Two mg. of each of the 2,4-D esters were administered intraperitoneally daily to groups of five C3H mice bearing transplanted

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mammary carcinoma. Tumor development and longevity of these animals did not differ materially from uninjected controls.

Slides were prepared from the tumors and other organs of Strain A mice bearing transplanted lymphomas and receiving varying amounts of 2,4-D daily. The pathological findings indicate more cellular destruction and the presence of more pyknotic cells in the tumors of mice receiving 4 mg. of 2,4-D daily than in those receiving lesser amounts or in uninjected controls.

b. Several fungicides were obtained from Dr. Pratt of the College of Pharmacy, University of California. These include (1) salicylacetone; (2) 2,4-dihydroxybenzophenone; (3) 4-4'-dihydroxydibenzolacetone; and (4) 2-methyl-4-amino-1-naphthol hydrochloride.

After gross toxicity studies, four mg. of salicylacetone, and 1 mg. of each of the other compounds were administered daily to groups of five Strain C3H mice bearing transplanted mammary carcinoma. Tumor size was determined at intervals. The tumor size and longevity of the experimental animals and of uninjected controls did not differ materially.

IV. Immunology.

1. The work has dealt chiefly with the fractionation of normal and tumor tissues obtained from human sources. The method was reviewed in the previous report. The preparation of the fractions and analyses have now been completed.

Several of these fractions have been used to induce antibody formation in guinea pigs and in rabbits. The lipid fractions extracted by benzene and ether from the sarcoma material were capable of inducing only

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non-specific reactions when tested in the Schultz-Dale apparatus by in vitro anaphylaxis. A more promising fraction from this sarcoma was a protein obtained by 20 percent methanol precipitation. It did not cross-react by in vitro anaphylaxis with normal liver or spleen fractions. Further work is being done with this fraction to establish its antigenic specificity. Rabbits have been injected with sarcoma, liver and spleen fractions; they will be bled for precipitins to support the findings with the Schultz-Dale technic. Rabbits were also immunized to fractions obtained from metastatic carcinoma of the cervix, as well as liver from the same patient. These animals were bled and their sera were tested by the precipitation reaction with homologous and heterologous antigens. There is some crossing between these two tissues in the fractions tested. Precipitin absorption experiments are under way to secure the antibody specific for the tumor antigen.

The anaplastic carcinoma of the lung was fractionated and its components are ready for injection of animals.

2. An attempt is being made to prepare radioactive antibody. The plan is to introduce radio-iodine-tagged gamma globulin into an animal previously injected with the homologous antigen to determine where the antibody localizes in vivo. This was done by Pressman and Keighley with anti-kidney serum in rats. To date rabbit anti-ovalbumin serum has been fractionated. The method used was that applied by Jager and Nickerson to human serum for routine clinical use. An electrophoresis analysis of this fraction shows it to be 60-80 percent gamma globulin. This globulin will be iodinated and tested in vitro by the precipitation reaction and in vivo in animals injected with ovalbumin.

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If this technic is satisfactory it will be carried out with some of the antibodies against specific tumor fractions in vivo.

3. Approximately 200 grams of transplantable mammary carcinoma of Strain C3H mice was homogenized and lyophilized. The dried tissue was split into 5 fractions: (1) fats; (2) pH 8.5 soluble material; (3) material precipitating at pH 4.2 from fraction (2); (4) 1 M-soluble fraction; and (5) residue. The fractions were injected into rabbits for 20 injections and a rest period of 10 days. The rabbits were then bled and the gamma globulin fractions extracted. These globulins will be radio-iodated in the near future and injected into mice with the mammary tumor to observe whether any localization will occur.

Respectfully submitted,

Michael B. Shimkin

Michael B. Shimkin, Surgeon
Director, Laboratory of
Experimental Oncology

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