TABLE 1

CHANGE IN COUNTING RATE WITH AGE OF SAMPLE, CORRECTED FOR DECAY

Set	Description of sample	Relative counting rate on successive days						
		0	1	2	3	4	5	7
1	1 ml of solution in Al cup kept in air (av of 2)	100	73	—	—	82	—	—
2	1 ml of solution in glass cup kept in air (av of 3)	100	106	109	111	115	—	125
3	1 ml of solution in glass cups kept in hygrostat over 1 *M* KSCN (av of 4)	100	104	105	104	104	—	106
4	5 ml of solution in glass beakers kept in air (av of 2)	100	98	98	100	—	—	—
5	1 ml of solution in glass cups kept in hygrostat over water (av of 7)	100	100	100	—	—	100	99

TABLE 2

RELATIVE COUNT CORRECTED FOR DECAY AND FOR DECREASE IN VOLUME

Set	Description of sample	Relative counting rate on successive days					
		0	1	2	3	4	7
2	1 ml of sample in glass cups kept in air (av of 3)	100	101	99	99	99	92
3	1-ml samples in glass cups kept in hygrostat over 1 *M* KSCN (av of 4)	100	102	103	101	101	102

caused by plating. Adsorption onto the lacquered surface was not thought to be significant, because of the presence of carrier and complexing agent, an assumption substantiated by the experimental results described below. When samples were weighed immediately before or after they were counted, an observed count could be corrected by a factor that took into account the increase in concentration resulting from loss of solvent through the dry film of lacquer. Set 2 in Tables 1 and 2 illustrates typical results before and after multiplying by a correction factor which is the percentage of original volume remaining at the time the sample was counted. As one might expect, the evaporation can be eliminated by using a hygrostat, as shown by Set 5. Occasionally, small droplets of water condensed on top of the film, but they can be removed easily without damage to the film by using an absorbent paper tissue. Ordinarily, the change in weight of a cup having a surface area of approximately 3.5 cm^2 was about -30 mg/day in the open air, -6 mg/day over 1 *M* potassium thiocyanate, and ± 1 mg/day over water. One can also decrease the evaporation error by using a large volume of sample without changing the surface area, so that the relative loss in weight is insignificant, as illustrated by Set 4.

These studies point out that in spite of careful coating of a metal container the plating of a more noble metal onto a less noble metal is very probable. More important is the fact that either the counting of liquid samples must be done within a few hours after preparation of the sample or the loss of solvent eliminated by placing the samples in a hygrostat after the lacquer film has dried. For best results, a correction should be applied for changes in the volume of the sample. Use of a hygrostat appeared to increase the average lifetime of the lacquer films from about 10 days in open air to about 3 weeks.

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A Volumetric Microrespirometer for Studies of Tissue Metabolism[1]

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A microrespirometer has been developed for studies of metabolism in small animals, tissues, cell suspensions, etc. It is based upon models of volumetric respirometers as constructed by Winterstein (*1, 2*), Scholander (*3, 4*), and Wennesland (*5*). From Scholander (*4*) has been adopted the use of a plastic block into which a V-shaped manometer has been drilled, connecting the respiration chamber with the compensating vessel.

New features are the inclusion in the plastic block of a chamber for oxygen replacement, and a new measuring device for the gas exchange. The latter has also been developed into a measuring and delivery burette for regular laboratory use (unpublished).

The apparatus is a constant pressure respirometer, maintaining the principal features of Winterstein's original model. The gases are kept under constant temperature and pressure, and the changes in volume are read directly. The theory is thus very simple: no vessel constants need be determined or calculated. The

[1] This work was supported by a Norwegian government scholarship and in part by contract N-6 onr 251-XIV between Stanford University and the Office of Naval Research.

[2] I wish to express my gratitude to John H. Lawrence, of the Donner Laboratory, University of California, Berkeley, and his staff for support and hospitality.

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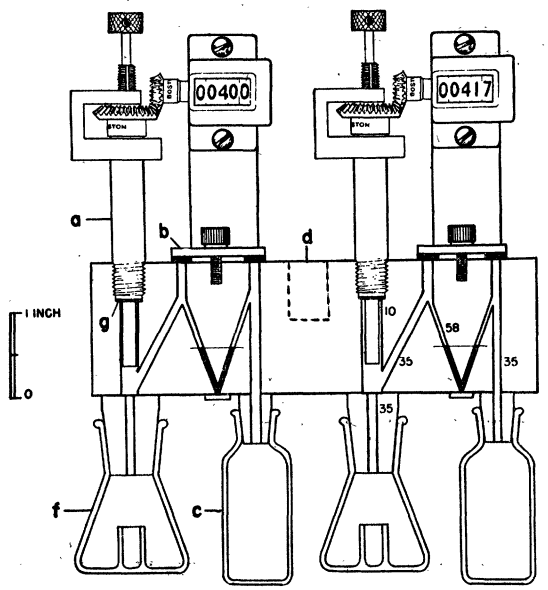

FIG. 1. One manometer block with two respirometer units.

only part to be calibrated is the volumetric device, which is uniform for all sets. Vessels of different types and sizes can be applied, and media of different quantity and composition used, without recalibrating the apparatus. The manometer necessary for adjusting the constant pressure is closed and connected to a compensating chamber, which makes it insensitive to changes in barometric pressure and humidity during the experiment, and much less sensitive to changes in the environmental temperature than an uncompensated system.

The apparatus (Fig. 1) consists of the following parts: respiration chamber; compensating vessel; Plexiglass manometer block, which also contains the oxygen delivery chamber; delivery and measuring device for oxygen; and mounting and shaking device.

The conventional Warburg flasks with one side arm and center well may be used for the respiration chamber (Fig. 1, *f*). I have made such flasks easily and cheaply from Plexiglass (Fig. 2 *A, f*).

For compensating vessels small bottles (Fig. 1, *c*) with standard ground glass stoppers (e.g., A. H. Thomas, Cat. No. 2232), of approximately the same volume as the Warburg flasks, may be employed. It is preferable to make the compensating chambers from Plexiglass. Their openings can be provided with screw threads to fit a threaded plug instead of the tapered one seen in Fig. 1. Plexiglass Warburg flasks can also be made with a threaded connection. If wanted, the threaded plug is converted into a tapered one by an adapter. The advantage of the threaded connection is the tight fit, which eliminates the need for such tightening devices as springs, rubber bands, etc., generally used to secure the tapered attachments.

The Plexiglass manometer block adapted for two respirometers is shown in Fig. 1. The figures to the right of the bores show the scale number of the drills used. After the apparatus has been thermoequilibrated, the manometer openings are closed simultaneously by a crossbar with two neoprene disks (Fig. 1, *b*). The bar is loosened and fastened by a binding post (Eby junior).

For the delivery and measurement of oxygen a simplified micrometer device is used (Fig. 1, *a*): the plunger and micrometer screw are made in one piece from a polished stainless steel rod of 3/16-in. diameter. The micrometer barrel can be made of Plexiglass or stainless steel. It is attached to the upper part of the oxygen chamber by a screw thread, and has a double gasket (*g*) of neoprene and vulcanized fiber material to give an airtight fit. The screw movement of the plunger is transferred through a set of bevel gears (Boston gear, Cat. No. G 479) to a commercial revolution counter (Veeder Roth square case revolution counter, Cat. No. A 114135). By choosing a micrometer screw thread of 32 to the inch, a gear ratio of 2/1 and the counter type mentioned, which records 10 units for each counter axis revolution, one inch displacement of the plunger gives 640 reading units ($32 \times 2 \times 10$). Each counter unit thus represents a volume displacement of .707 μl.

The mounting and shaking device is shown in Fig. 2. (I am indebted to Professor J. M. Crismon, of the Physiology Department at Stanford University, for

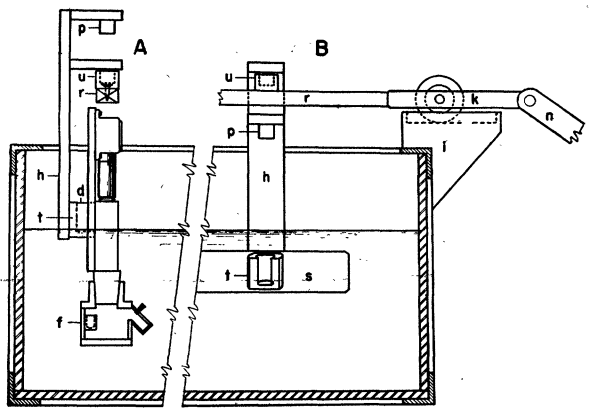

FIG. 2. *A*, side view of mounting device and shaking rod with one respirometer attached, suspended in the upper position with manometer bores above the water surface. *B*, right end of the same without respirometer, suspended in the deepest position. (Front view.)

valuable suggestions.) The respirometer blocks can be attached individually to the shaking rod by a double suspension hook of Plexiglass (Fig. 2 *A, h*), or the receptacles (*t*) can all be cemented to a strip of Plexiglass (*s*), each end of which is suspended from the shaking rod by similar hooks (Fig. 2 *B, h*). A modified dovetail arrangement is used for the mounting. The male parts (Figs. 1 and 2 *A, d*) are made from pieces of ½-in. Plexiglass rod, of which a segment is milled off to provide a face for cementing it to the back of the manometer block. The receptacles (Fig. 2, *t*) are made from a ¾-in. rod, into which a hole of ½-in. diameter is drilled, and 2 parallel faces

milled off, one corresponding to the segment milled off from the male part, the other for cementing the receptacles to the mounting strip. The suspension hooks rest by small plugs (*p*) in cups (*u*) mounted on top of the aluminum shaking rod (*r*). The rod is extended across the water bath, and has a small ball-bearing carriage (*k*) at each end, which rolls in a shallow groove of a rig (*i*) attached to the edges of the water bath. It is driven by a crank (*n*). The amplitude and rate of shaking are of the same order as with the conventional Warburg apparatus.

The only calibration necessary is to measure the diameter of the plungers by means of a commercial micrometer caliper. For very accurate work this should be done at the experimental temperature. The feed of the micrometer screw should be checked against a micrometer caliper head.

All parts are greased with a chemically neutral grease, such as Nevastane heavy X (Keystone). The compensating vessels should contain a few drops of water. With a long syringe needle the manometers are filled to the level line with the manometer fluid; e.g., water containing a little detergent and some dye such as T 1824, or kerosene with a little Sudan IV. The tissue or other respiring material is placed in the respiration chamber, which contains the medium and in the center well about 0.2 ml 5% KOH. The chamber can be flushed with oxygen through the side arm. After the side arm has been closed, the mounting strip with the respirometers is attached to the shaking rod in the upper position (Fig. 2 *A*) with the manometer openings above the water. It should be shaken 10–15 min for thermoequilibration. The manometer openings are closed, and the mounting device is moved to its deepest position (Fig. 2 *B*), so that all gas volumes are under water. After shaking another 10 min, the manometers are adjusted to the level line, and the initial reading is taken. At regular intervals the manometers are brought back to balance and readings are made.

The difference between consecutive readings is the oxygen consumption for that interval, uncorrected for standard temperature and pressure. Each counter unit corresponds to .707 µl. Suppose, in an experiment measuring oxygen consumption, the average difference between readings taken at intervals of 15 min is 32 counter units: The oxygen consumption for that period has been $32 \times .707 = 23.6$ µl of O_2 at the temperature of the experiment, and barometric pressure as observed at the time of the closure of the manometers. The figure is converted into volume O_2NTP per unit of tissue weight per hour in the usual way.

Blank runs have given an oxygen consumption of 1–2 counter units per hour, which is generally negligible.

For further details as to the construction of the respirometer, operation, etc., I refer to the description of the previous model in Umbreit (*5*), and to Peiss and Wennesland (*6*). As the principal features are retained, the experiences of Peiss and Wennesland are directly applicable to the present model: The

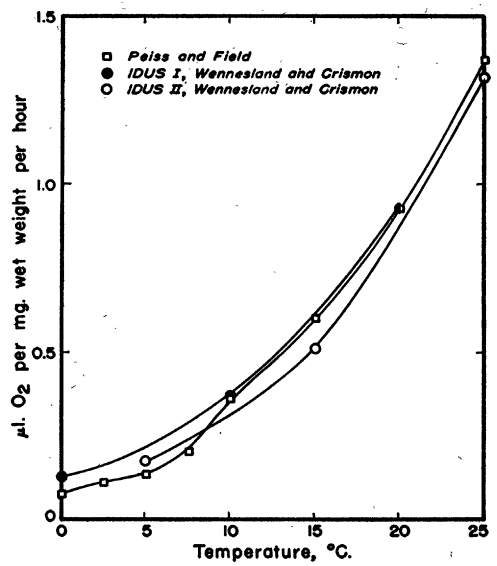

FIG. 3. Oxygen consumption of brain mince of the Golden Orfe (*Idus melanotus*) adapted to 25° C tested at temperatures from 0° C to 25° C. Data from Peiss and Field's work performed with the Warburg apparatus (137 observations) compared to two groups (Idus I and II) tested with the present respirometer (89 observations). O_2 consumption in µl/mg wet wt/hr.

oxygen consumption of rat brain cortex slices was tested at 37.5° C, 3 aliquants by the Warburg method and 3 by the volumetric apparatus. In all, 48 Warburg runs were made and 47 volumetric (one sample lost). The mean Q_{O_2}s were 14.12 and 14.08, respectively. The corresponding standard errors were ± 0.169 and ± 0.171. Statistical analysis showed that the two series did not differ significantly either in respect to the means (Student's *t* test) or of the variability (Fisher's *F* test).

The present model has been used for more than a year in an extensive series of studies by Wennesland and Crismon (*7*) of brain tissue metabolism of fish at temperatures between 0° C and 25° C. Figures from two groups of the Golden Orfe (*Idus melanotus*) adapted to 25° C compare favorably with corresponding experiments published recently by Peiss and Field (*8*), who used the Warburg method. In Fig. 3 the curves from the two groups of *Idus* experiments (called Idus I and II) are compared with the corresponding curve drawn from the figures in Peiss and Field's paper.

The range of the apparatus can be extended downward and upward by changing the dimensions. I have made respirometers with a sensitivity of 0.05 µl, and a large modification which allows a total gas displacement of about 6 ml divided into 1,280 counter units. Because of the size of the chamber of the latter construction the manometer block and compensation chamber were cemented on top of a circular base plate. The respiration chamber was attached underneath by a screw clamp device.

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A New Common Biochemical Property of Tumors Derived from Different Tissues[1]

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Previous investigations from this laboratory have demonstrated that polarographically reducible materials present in the epidermis of the mouse and man and in the liver and muscle of the mouse are structurally altered when these tissues become malignant (*1–3*). The reducible materials also absorb in the ultraviolet. Evidence of an alteration in the structure of the reducible material in the malignant transformation of epidermis to squamous-cell carcinoma was given by differences in the half-wave potentials and in the absorption characteristics in the ultraviolet of the material from epidermis as compared to that from the carcinoma (*3*). The data presented in this report further substantiate our previous results on a qualitative chemical change in carcinogenesis, and they also show that the tumors examined have a common biochemical property resulting from this alteration.

Methods for the extraction and partial purification of the reducible materials have been given (*3*). Briefly, the tissues were extracted with mixtures of alcohol and peroxide-free ethyl ether, and the total lipid thus obtained by evaporation of the solvents was re-extracted with dry ether, filtered, and the ether removed on a steam bath. Then the acetone soluble fraction of the total lipid, which contained the reducible material, was further fractionated by partitioning it between alcohol, acetone, and water saturated with petroleum ether against the latter saturated with alcohol, acetone, and water. The polarographically reducible material obtained in this manner represented 0.01–0.02% by weight of the fresh tissue. Then nonreducible compounds containing phosphorus were precipitated from the partially purified material in an alcohol-water mixture with calcium chloride (*3*). The latter was spun down at 0° C at 2,500 rpm, and the supernatant was dried at 56°–60° C in a vacuum oven. The dry residue was then treated with 4–6 ml ice-cold water, from which a colored nonreducible substance was separated by centrifugation at 0° C. If the reducible material at this stage was highly colored, much of the colored material could be removed by several extractions with 10-ml portions of peroxide-free ether. The reducible materials thus obtained are light-yellow in color, hygroscopic, soluble in water, alcohol, *N* butyl alcohol, *N* amyl alcohol, and only slightly soluble in nonpolar solvents. The materials are dialyzable through cellophane, stable to heat (steam bath), to storage at 0° to 4° C for a period of months and to oxygen. They appear to be nonprotein.

[1] This investigation was aided by grants from the Charles F. Kettering Foundation and the American Cancer Society.

Some of the polarographic data obtained from the reducible materials are of interest since they may aid in determining whether the reduction is reversible, and in establishing the number of electrons involved in their reduction (*4*). Although the first wave of the double wave of the material from normal and hyperplastic epidermis (Table 1) appeared to be diffusion-controlled, since the diffusion current and the half-wave potentials were independent of the buffer used at constant pH, from pH 4.0 to 7.2, the relationship between i_d, the diffusion current, and h, the height of the mercury reservoir, was determined. For this experiment the purified material from hyperplastic epidermis was dissolved in 1.5 ml dioxane, 1.5 ml citrate buffer (0.1 *M*) of pH 3.16 (final pH, 4.2), and sufficient tetrabutylammonium iodide was added to make the solution 0.1 molar. The results are shown below:

h(Hg)	i_d(μA)		$i_d/h^{1/2}$
40.5	1.68		0.264
50.5	1.80		0.253
60.5	1.99		0.256
70.5	2.19		0.263
		Average	0.259

Since $i_d = Kh^{1/2}$, the reaction at the dropping mercury electrode is diffusion-controlled (*4*). Similar data were found for the material from the squamous-cell carcinoma.

The diffusion currents and the half-wave potentials were determined on the material from normal epidermis at 2°, 15°, and 25° C in citrate buffer, dioxane, and tetrabutylammonium iodide mixture of pH 6.4, and from liver in the same mixture buffered at pH 5.2 at 2°, 25°, and 40° C. From a plot of the diffusion current against the temperature, the slope of the straight line for the material from normal epidermis gave a temperature coefficient of 1.4%/degree; that from liver was 3.0%/degree. These coefficients are of the same order of magnitude as that of normal diffusion currents (*4*). Furthermore, the half-wave potentials were independent of the temperature, which may indicate a reversible reaction at the dropping mercury electrode (*4*).

In another set of experiments the materials from normal epidermis, squamous-cell carcinoma, liver, hepatoma, and rhabdomyosarcoma were polarographed as previously described (*2*) and log $i/(i_d - i)$ was plotted against E_{de}, the potential at the dropping mercury electrode (*4*). The reciprocal of the slope of the straight lines from the materials from these tissues