price of commercially available models, which provide, as a rule, only one testing condition. The device may also be used as a square wave stimulus generator, for nerve and muscle physiology, replacing the glow modulator tube by coupling capacitor and potentiometer to chassis ground.

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A Method for Preparing Slide Mounts of Small Invertebrates

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For some years the writers have been working on methods of mounting insects and other small invertebrates on slides for permanent preservation. Basically the procedure finally adopted follows that suggested by Middlekauff (*1*), but several major modifications have been made to adapt it to different needs. Furthermore, the method here presented has been found excellent for mounting both immature and adult insects, as well as other arthropods, helminths, and so on.

Adult insects are collected in 95% ethyl alcohol. Soft-bodied stages that are subject to shrinkage and discoloration are collected in a killing fluid recommended by Alvah Peterson and composed of 95% ethyl alcohol, 10 parts; glacial acetic acid, 2 parts; kerosene, 1 part; and dioxane, 1 part. Larvae should be kept in the solution until properly distended and then placed in 95% ethyl alcohol. For most adults, no special killing fluid is necessary or even desirable. It is important to note, however, that in many instances, color features may be preserved by mounting immediately after collecting. This is true, for example, of some greens in adult midges, which ordinarily fade considerably. Helminths fixed by usual laboratory methods can be washed and stored in 95% ethyl alcohol. Formalin-preserved specimens must be washed carefully and carried up to 95% alcohol before utilizing this method.

To prepare specimens, use three 5-ml beakers containing 95% alcohol, absolute alcohol, and beechwood creosote, respectively. Total volume of the specimens processed should not exceed about 10% of the volume of the fluid. Specimens are left in 95% alcohol a few minutes, in absolute alcohol at least a minute, and then transferred to creosote, where they invariably float; they should be retained in it until they sink to the bottom of the beaker. This may take 10 min or longer. Specimens may be kept in the creosote indefinitely without apparent harm. Ticks, other arthropods, and helminths have been stored in creosote for 12–15 months prior to their preparation as permanent mounts. After several hours in the creosote, specimens may be mounted whether they sink or not, although failure to sink is usually indicative of the presence of air spaces in the specimen, and such specimens are not likely to make the best mounts. Some nematodes require an immersion in a fourth 5-ml beaker containing an equal mixture of beechwood creosote and diaphane, to facilitate the penetration of the mounting medium into the body spaces of the specimens. Before transferring specimens to a glass slide, a drop of diaphane is placed on the slide. Specimens are moved from the creosote to the diaphane droplet, covered with more diaphane, and then covered with a cover slip. Mounts should be examined daily, and diaphane added until air spaces cease to develop under the cover slip.

If specimens are thick enough to cause a noticeable tilting of the cover slip, clear celluloid or plastic supports, about 3 × 6 mm, may be placed on the slide so as to hold the cover slip in a perfectly horizontal position. If materials of several different thicknesses are available, the correct thickness may be selected for the specimen at hand, but very thin supports may be used for all purposes by creasing them across the middle. Such creased supports have a desirable degree of springiness.

Perhaps the principal advantages of the method here described are its simplicity and broad applicability.

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Roentgen Irradiation of Para-aminobenzoic Acid Solutions

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Recent progress in radiation therapy of neoplastic diseases has focused attention on the radiation chemistry of the action of ionizing radiations on aqueous solutions of biologically significant organic compounds (*1*). Studies on certain amino acids, vitamins, enzymes, nucleotides, proteins, and steroids have already been reported. Dosages of radiations, however, were much greater than those therapeutically employed, whereas concentrations of solutions used were generally much higher than those obtained under physiological or therapeutic conditions. Studies under conditions more closely approximating those found clinically may yet prove steppingstones to the

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elucidation of the chemotherapeutic action of these radiations on neoplastic tissues.

During the past decade or so, *p*-aminobenzoic acid (PABA) and its derivatives have occupied the limelight in the field of chemotherapy. Certain clinical observations indicate an intimate relationship between the pharmacological action of these compounds and their radiochemical behavior. Patients receiving combined sulfonamide and x-ray therapy often suffer severe reactions not produced if either treatment is given separately (*2,3*). One of us (I.C.C.T.) has previously shown that certain sulfonamides are broken down *in vitro* by x-irradiation (*4,5*). These breakdown products have not yet been identified but may play a part in such toxic reactions. On the other hand, PABA, the mother substance of these compounds, has been used to counteract the effects of solar irradiations (*6,7*). It has been our experience that, by combining PABA with x-ray therapy, a more potent effect from the radiation on malignant growths is obtained than if x-rays are used alone. Hematological observations indicate a probable relationship with folic acid (unpublished results). These interesting observations, together with the fact that no reference can be found to any investigation on the radiation chemistry of PABA and its derivatives (other than the above-mentioned preliminary report), prompted a reinvestigation of the study interrupted by the war. Preliminary results are reported here.

Stock millimolar solutions of PABA[1] were prepared fresh each week, and dilutions made up therefrom immediately before use. Triple-distilled water was employed for all solutions. Matched Pyrex test tubes (20 × 150 mm, wall thickness, *ca* 1 mm), each containing 25 ml PABA solution, were irradiated in batches of 16 tubes arranged in 4 rows, each 4 tubes deep, and held in place by a square wooden holder. All tubes were uncorked during irradiation.

Preliminary trials made with a 400-kv G-E maximar machine yielded irregular results. Data reported hereunder were obtained with a 250-kv constant-potential Westinghouse therapy machine at 230 kv, 15 ma, with no added filter and with the front of the test-tube holder 22 cm from the target. The time of irradiation was controlled by an automatic timer, and dosages received by each tube were measured with a Victoreen condenser r-meter.

Immediately after irradiation, the amount of PABA remaining in solution was estimated in triplicate on 2.0-ml samples taken from each tube. A modified Bratton-Marshall's test (*8*) was followed, using *N*(1-naphthyl-)ethylenediamine hydrochloride as coupling agent, and the resulting color was estimated on a Beckman DU spectrophotometer at wavelength 535 mμ.

Fig. 1 summarizes graphically the data obtained for three series of experiments with solutions containing 10 mμM (1.37γ)/ml. Percentages of PABA

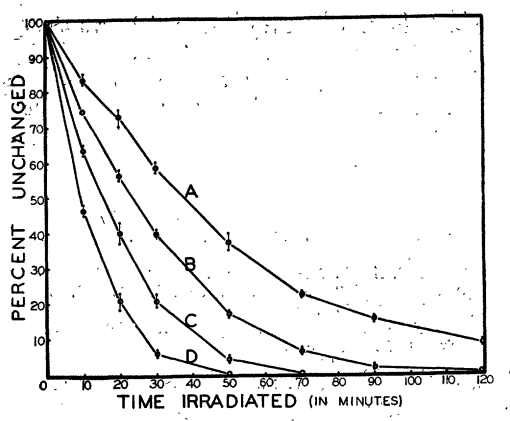

FIG. 1. X-irradiation of *p*-aminobenzoic acid, showing percentage unchanged in 25 ml aqueous solution (10 mμM/ml) at dosage rates of curve *A* 400 r/min, *B* 640 r/min, *C* 1,000 r/min, and *D* 1,650 r/min.

unchanged are plotted against their respective period of irradiation. The time factor is used, since accurate measurement of the actual dosages received in terms of roentgens was not possible, because of intensity of irradiation—the time for discharge of the meter was so short (10 sec) that accurate readings could not be made. The dosages listed under the caption of Fig. 1 represent the average of these readings from the 4 tubes in each row parallel to the axis of the x-ray tube. Curve *D* represents the rate of destruction of PABA in the front row of tubes, Curve *C* the second, etc. With solutions of higher concentrations (50–100 mμM/ml), similar curves were obtained—the higher the concentration the flatter the curve. Lower concentrations were not studied, since they do not lend themselves to chemical estimation by the Marshall test.

Since the characteristic color of Marshall's test is developed only in the presence of a free amino group attached to the benzene nucleus, the test is at least a measure of the cleavage of the amino group from the parent compound and, therefore, a gauge of the destruction of the PABA molecule. Browning of the solutions was noticed, but no precipitate was obtained, even on long standing. A saturated solution on prolonged irradiation yielded a small amount of brown precipitate, the nature of which has not yet been determined. Ultraviolet absorption curves of solutions irradiated for varying lengths of time yielded only the characteristic PABA curve, with the longer the time of irradiation, the flatter the curve. Solutions giving a negative Marshall's test exhibited no absorption in the ultraviolet.

It will be seen that PABA at dilutions approximating those usually found under physiological and therapeutic conditions in human tissues is destroyed by x-irradiation in an exponential manner. This verifies the preliminary observation made by one of us (I.C.C.T.) in a former paper (*4*). We have not been able to determine what chemical change took place in the PABA molecule on irradiation. Microchemical and spectrochemical studies on the products of the

[1] We are indebted to Merck & Co., Inc., Rahway, N. J., for a generous supply of especially purified crystals of PABA for these experiments.

reaction have so far yielded inconclusive results, probably because of the high dilutions employed. Further studies are in progress, following the recent reports of Weiss *et al.* (*9*), which appeared while the present study was being carried out.

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The Decrease of Critical Flicker Frequency with Age

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The perception of flicker and fusion is one of the most interesting visual phenomena. In recent years considerable interest has been shown in the measurements of critical flicker frequency (cff), which have been used in many studies. Therefore, knowledge of all the factors determining cff has become of great theoretical and practical importance. A number of investigators have pointed out age as a factor in cff. Some investigators, such as Meili and Tobler (*1*), G. W. Hartmann (*2*), and V. L. Miller (*3*), comparing different age groups of children and young adults over a relatively narrow age range, have not found any change in cff with age. Investigators who studied larger age ranges, however, such as Simonson, Enzer, and Blankstein (*4*), Brožek and Keys (*5*), Weekers (*6*), and Misiak (*7*), reported a significant decrease in cff in older age groups. The results of their investigations have not been conclusive because:

1. They were based on relatively small numbers of Ss.
2. They failed in having a sufficiently wide age range.
3. The results were not comparable, since they were obtained by different methods.

From these studies nothing definite could be inferred concerning the form, extent, and cause of the decrease of cff with age. Thus it was thought to be of value to measure cff of sufficiently representative samples over a large age range, using the same apparatus and procedure throughout the study.[1]

The subjects were 182 males and 137 females free from any visual pathology, ranging from 7 to 89 years of age, with a mean age of 36 years. After the completion of the study, they were divided into 17 five-year groups and their cff's and variability compared.

With a few minor changes, the apparatus used in this study was built from the specifications and data presented in an article by F. Henry (*8*). It was an electronic apparatus which had a 3-w neon glow lamp giving an intermittent light at a rate determined by 3 adjustable resistors. Another neon lamp, similar to the lamp producing the flicker, served as a voltage regulator, thus assuring the constancy of the flicker rate, even if the supply voltage varied. A small cathode-ray oscilloscope tube built into the apparatus permitted its calibration. The circular test patch was 5 mm in diameter. From a distance of 12.8 in. the subtending visual angle was 48 min, assuring foveal observation of flicker when the eyes were fixated on the test patch.

All the Ss were adapted to the illumination level of the light source of the flicker apparatus before they were tested for cff. The observations were made with the dominant eye. The frequency of light flashes was gradually increased until the subject reported no flicker, and the frequency was then decreased until the flicker was again seen. The cff value of each S was the mean of the readings obtained from flicker to fusion and from fusion to flicker, which numbered 20 on the average. In the early stage of the research, Ss were tested for 10 days with the number of readings ranging as high as 80, but when no significant day-to-day changes were found, and the correlation coefficient between readings obtained on successive days was above .95, the number of readings was reduced.

The group results are presented in Table 1. The mean cff under the experimental conditions of the study was 41.18 cps for the males, 41.08 for the

TABLE 1

MEANS AND VARIABILITY OF CRITICAL FLICKER FREQUENCIES FOR DIFFERENT AGE GROUPS

Age groups	N	Cff	Inter-individual variability SD	Intra-individual variability AD
7–11	35	42.91	4.31	.90
12–16	36	44.85	4.36	.71
17–21	39	43.63	3.54	.58
22–26	39	42.62	3.86	.83
27–31	16	42.65	3.51	.62
32–36	26	40.86	3.64	.79
37–41	18	40.27	4.07	.74
42–46	14	40.42	3.98	.92
47–51	18	39.46	3.90	.75
52–56	13	40.99	6.15	.60
57–61	12	38.38	3.42	.77
62–66	5	36.06	2.10	.35
67–71	14	38.91	5.46	.35
72–76	13	36.41	3.81	.33
77–81	13	32.22	6.25	.36
82–86	6	35.82	5.69	.37
87–91	2	36.04	3.61	.47
Total	319			
Mean		41.14	4.22	.68

[1] This undertaking was carried out through the help of I. McCormick, J. Doblmeier, and W. McGill, of Fordham University, who made their results available to the author and to whom he expresses his thanks and appreciation.