REFERENCES

- Berenblum, I. & Kendal, L. P. (1936). *Biochem. J.* **30**, 429.
 — & Schoental, R. (1942). *Biochem. J.* **36**, 86.
 Bryan, W. R. & Shimkin, M. B. (1943). *J. nat. Cancer Inst.* **3**, 503.
 Chalmers, J. G. (1934). *Biochem. J.* **28**, 1214.
 — (1938). *Biochem. J.* **32**, 271.
 — (1940). *Biochem. J.* **34**, 678.
 — & Peacock, P. R. (1936). *Biochem. J.* **30**, 1242.
 Clarke, H. T. & Rahrs, E. J. (1923). *Industr. engng Chem.* **15**, 349.
 Dickens, F. & Weil-Malherbe, H. (1942). *Cancer Res.* **2**, 560.
 Hieger, I. (1936). *Amer. J. Cancer*, **28**, 522.
 Jones, R. N. (1942). *Cancer Res.* **2**, 237.
 Lorenz, E. & Shear, M. J. (1936). *Amer. J. Cancer*, **26**, 333.
 — & Shimkin, M. B. (1941). *J. nat. Cancer Inst.* **2**, 491.
 — & Stewart, H. L. (1940). *J. nat. Cancer Inst.* **1**, 17.
 Peacock, P. R. (1936). *Brit. J. exp. Path.* **17**, 164.
 Weil-Malherbe, H. (1943). *J. chem. Soc.* p. 303.
 — (1944). *Cancer Res.* **4**, 102.
 — & Weiss, J. (1942). *Nature, Lond.*, **149**, 471.

A Note on the Fluorimetric Estimation of 3:4-Benzpyrene by the Method of Miller and Baumann

By H. WEIL-MALHERBE, *Cancer Research Laboratory, North of England Council of the British Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle-on-Tyne*

(Received 7 March 1944)

After completion of the preceding paper (Weil-Malherbe, 1944*b*) a communication by Miller & Baumann (1943) on the fluorimetric estimation of carcinogenic hydrocarbons came to hand. These authors describe an ingenious method of 'two-condition fluorimetry' in which use is made of the different susceptibility to the quenching effect of O_2 of hydrocarbons on one hand and tissue material on the other (cf. Weil-Malherbe, 1944*a*). If the quenching ratios of both are known, the composition of a mixture can be deduced from the increment of fluorescence obtained on changing from an oxygenated to an oxygen-free medium.

The method has been tested in the following experiment with amounts of 0.2, 0.3 and 0.5 $\mu g.$ benzpyrene added to three previously purified mouse extracts. Benzene was used as solvent and the measurements carried out first in pure N_2 and then in pure O_2 . The fluorescence ratios (cf. Miller & Baumann, 1943) were: $N=8.8$, $n=3.8$, K ($\mu g.$ benzpyrene/unit of fluorescence in O_2) = 0.032.

Benzpyrene added ($\mu g.$)	Reading in N_2	Reading in O_2	Benzpyrene recovered ($\mu g.$)
0.2	227	51	0.21
0.3	218	45	0.30
0.5	272	52	0.48

These results are very satisfactory and there is no doubt that the method of Miller & Baumann will extend the range of sensitivity of the fluorimetric method considerably.

One point of Miller & Baumann's method could not be confirmed. They use crude, unpurified extracts directly for the fluorimetric analysis. These crude extracts are, at any rate as far as whole mice are concerned, entirely unsuitable for fluorimetry for the following reasons: (1) They very often contain finely suspended matter which cannot be re-

moved by centrifugation or which reappears on standing after centrifugation. This is especially true for unchilled extracts. (2) They usually contain yellow pigments of animal origin, and other material, which exert a filter effect by absorbing either the primary or the secondary light. Their fluorescence is white or yellow with hardly any blue component; thus with a violet or blue colour filter the paradoxical result is obtained that they cause a smaller reading than the purified extract. Addition of benzpyrene to these crude extracts does not result in a corresponding increase of fluorescence, as shown by the following experiment:

Extract	Dilution*	Colour	Reading before and after the addition of 1 $\mu g.$ benzpyrene	
			Before	After
None	—	—	—	118
1	1 in 20	Colourless	0	71
2	1 in 2	Colourless	13	61
3	1 in 2	Yellow	0	4

* Compared with the concentration used for fluorimetry on purified extracts.

It follows (1) that yellow extracts may blot out the fluorescence of benzpyrene completely, (2) that even colourless extracts may reduce the fluorescence appreciably, and (3) that they may do so even after twentyfold dilution. It is unlikely that the technique of hydrolysis and extraction used by Miller & Baumann for large amounts of tissue, which is practically identical with that described in the preceding paper, would have yielded less impure extracts. It seems, therefore, that Miller & Baumann's method, if applied to whole mice, does not dispose of the necessity of purifying the extracts.

REFERENCES

- Miller, J. A. & Baumann, C. A. (1943). *Cancer Res.* **3**, 849.
 Weil-Malherbe, H. (1944*a*). *Cancer Res.* **4**, 102.
 — (1944*b*). *Biochem. J.* **38**, 135.