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QUANTITATIVE BETA TRACK AUTORADIOGRAPHY WITH NUCLEAR TRACK EMULSIONS

BY

HILDE LEVI AND ANNE S. HOGBEN

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The applicability of the nuclear track plate NTB-2 (of Eastman Kodak, Rochester, N.Y.) to quantitative beta track autoradiography has been investigated. A constant source of C-14 beta particles was brought in contact with the emulsion and the number of tracks registered per unit area of the plate was counted. The counting results obtained by four different investigators evaluating a total of 50 images were subjected to statistical analysis. The reproducibility of the track count was studied, both with respect to the counting ability of one and the same investigator repeatedly confronted with a given track pattern, and to the evaluation of the same pattern by different investigators. A statistical analysis of the counting results permits one to estimate the homogeneity of the emulsion in small areas of the plates, to compare the homogeneity of plates from the same batch, and their sensitivity from batch to batch. The efficiency of the plates and the effect of storage conditions have likewise been investigated. The contributions of various sources of error to the uncertainty of the final counting results were estimated.

Introduction.

In 1950, it was shown by Boyd and Levi[1] that beta particles of low energy, e. g., from radio-carbon or radio-sulphur, register as tracks in nuclear emulsions of the type NTB-2, manufactured by the Eastman Kodak Company in Rochester, N.Y. At that time, this was the only photographic emulsion commercially available which would register low energy electrons as tracks. This offered a possibility for improvement of the sensitivity and the resolution of existing autoradiographic techniques. In random grain autoradiography with low energy beta particles, on the average one grain is made developable per particle traveling through a thin emulsion. Therefore, at low level of activity, the blackening of the emulsion has to be established by grain counting. In the case of the NTB-2 emulsion, beta particles entering the emulsion produce tracks which are more easily recognized and counted than are single grains.

The mentioned authors floated sections of tissue containing a C-14 labeled compound on NTB-2 plates and observed—after suitable exposure—beta tracks in the emulsion under the tissue. The number of tracks per unit area under a section of a given thickness was counted and, besides, the amount of C-14 present in the tissue was determined by means of an ionization chamber. The track count was found to be in satisfactory agreement with the result of ionization chamber measurements on samples of the same tissue. These findings suggested that track autoradiography might be a means of localizing the labeled compounds within the tissue or the individual cells quantitatively and with a resolution higher than that obtained in a random grain autoradiogram on an NTB plate. However, it remained to investigate how high a resolution can be obtained in a beta track autoradiogram; evidence had to be presented that track counting offers a reproducible and quantitative measure of the amount of tracer element present in the tissue under investigation.

In order to verify the applicability and reproducibility of the method, standard conditions for exposure and processing of the plates, and a reproducible technique of track counting under the microscope had to be established. The development of the counting technique brought to our attention so many points of significance that some details of the method are described in the following.

After a number of unsuccessful attempts at preparing a suitably thin and homogeneously active standard which could easily be handled, it was decided to use as a standard a piece of a C-14 polystyrene foil (manufactured by the A.E.C. in Oak Ridge), containing 0.6 microcuries of C-14 per sq. cm. The foil weighs 28 mg/cm^2 and thus represents an "infinitely thick layer" of C-14.

Standard Exposure and Processing.

About 1 sq. cm. of the polystyrene foil was used as a standard in all cases described below. This standard was laid on the NTB-2 plates and covered with a piece of clean glass on which a 50 g load was placed. The exposure time was either 50 min., 100 min. or 150 min. throughout, because exposure for more than 150 min. would give rise to too many tracks per field (cf. below).

The NTB-2 plates were developed for 8 min. in a D-19 developer at 19—20° C, then fixed for 30 min. in 30 $^0/_0$ hypo, and washed for approximately one hour in tap water. The plates were rinsed in distilled water and dried in a horizontal position at room temperature. Finally, the exposed area on each plate was covered with balsam and a cover glass, 150—175 microns thick.

Track Counting Technique.

The tracks were observed in a Leitz Ortholux microscope, transmitted light, with 10 × oculars and a 45 × objective. One ocular contained a square net micrometer (eyepiece reticulum) subdivided into a hundred small squares. The field circumscribed by the square net was in most cases 125 × 125 square microns.[1]

It was found impossible accurately to localize a field to be counted by vernier readings of the movable stage alone. If not characterized by typical landmarks, a given field could not be found again with certainty after the plate had been moved on the stage. Also, there was no definite proof that the position of the plate under the microscope remained exactly the same during counting of a given field. In order to compare, for example, the counts obtained by different investigators, it was found necessary to establish accuracy of placement within a few microns and, therefore, characteristic landmarks were used as points of reference. Every plate registers a few alpha tracks, the end points of which may serve for exact orientation. Since the plastic box used for exposure was contaminated with traces of an alpha emitter, as a rule about 10—20 alpha tracks were registered within 1 sq. cm. of the image (and a similar amount in other areas of the plates).

Before the fields to be counted were chosen, the boundary of the image was measured up on the stage of the microscope and a ten times enlarged picture of the image was drawn. The positions of a number of alpha tracks located in the central part of the image (at least 500 microns from the edge) were chosen for orientation of the fields to be counted. One end of an α-track can be brought to coincide with one corner of the square reti-

[1] In the course of this study, three different microscopes of the same type, and with corresponding optics, were used. It was discovered later that the field sizes were not identical. Uncertainty as to the actual field size during counting of all series-1 plates has caused us to omit this series in the comparison between batches.

culum to an accuracy of 1 micron in either direction. In the same way, background fields situated several millimetres away from the image and to all four sides of the image, were identified by landmarks.

The choice of fields for counting within a given image is random, as the occurrence of the landmarks is random. The question remains, however, whether each landmark should serve to locate one field or whether—at the most—it can be used to describe the position of four adjacent fields. The result of different groupings is discussed below.

The most convenient and reproducible method of counting is to subdivide the field under observation into ten rows of ten small squares and to count one row at a time. If the number of tracks per field is low—as in a background field or after a 50 min. exposure of our standard—it will take an experienced investigator between 5 and 15 min. to count one field. As the number of tracks increases, it becomes increasingly cumbersome to count (15—30 min./field with 50—100 tracks/field). Also, at high track density, it becomes very difficult to disentangle the complex track patterns.

Identification of a "Track".

Electron tracks in photographic emulsions are characterized by their irregular zig-zag path. Moreover, it is typical of these tracks that the grain spacing in the beginning of the tracks is wider than towards their end, when the particles have given off most of their energy by collision. The tracks observed in the NTB-2 emulsion consisted on the average of six grains, tracks up to ten grains long did occur.

A photographic emulsion always shows some developed grains—fog or background—even if the emulsion has not been exposed to irradiation from a radioactive substance. Some of this background which is due to cosmic radiation will appear as electron tracks similar to those produced by carbon-14 betas. Fog due to chemical or mechanical effects, however, appears as randomly distributed grains. With increasing fog, there is thus an increasing probability for three or more grains to fall "in a line", simulating a track. It was decided to consider four grains in a line a minimum requirement for the track pattern. Thereby, the much more frequent accidental groups of three grains are not included in the track count. On the other hand, the shortest tracks produced by low energy beta particles will thus not be counted. Because of the shape of the energy distribution curve for the beta spectrum of radio carbon, the track count will be only a few per cent too low due to the mentioned minimum requirement.

Outline of Special Problems.

Besides the efficiency of the NTB-2 plates, i. e., the number of tracks registered from a source of given strength, and the relation between track count and exposure time, it is of paramount interest to investigate the reproducibility of the track count on different plates from the same batch and from different batches. Finally, external conditions during storage may affect the properties of the plates and ought to be investigated.

Obviously, however, an interpretation of results obtained by visual counting of tracks under the microscope must be based on a careful investigation into the reproducibility of the data obtained in repeated countings of a given field by one and the same investigator, and by different investigators confronted with the same field. This check was made throughout the present study; the results obtained were considered so important that they are presented in some detail. They demonstrate very clearly that the study of the properties of the plates is intimately connected with a study of the counting ability of the investigator. An attempt has been made to determine the relative sizes of the errors arising from the various sources.

Results.

Eastman Kodak plates of the type NTB-2 from five different factory batches, received in five shipments over a period of two years, were studied. The batches are designated, in order of receipt, series 1 to series 5.

The analysis of the results of track counts (see Tables I and II) is based on the counting data of four different investigators, denoted as A-B-C- and D. Only one of them, investigator B, has counted fields from all images included in the study. Investigators

Table 1.

Counting Data from Plates of Series 1 and 2 (cf. the text on pp. 13 and 14).

1 Series	2 Plate	3 Days between arrival and exposure	4 Exposure time in min.	5 Number of exposed fields counted	6 Mean exposed count per $(100)^2\,\mu^2$	7 Net mean count per $(100)^2\,\mu^2$	8 S. E. of net mean count per $(100)^2\,\mu^2$
1	10	2	50	13	32.9	22.0	2.97
1	15	3	50	28	34.5	24.5	1.19
1	3	1	100	32	65.4	51.0	3.29
1	13	3	100	36	67.1	55.5	4.77
1	5	1	100	7	85.3	69.4	5.12
1	17	4	100	6	74.5	62.1	3.72
1	18	4	100	7	88.4	78.8	4.94
1	19	4	100	5	69.4	57.7	4.73
1	11	2	150	24	112.0	99.3	7.49
1	12	2	150	6	101.1	88.4	5.15
2*	19	2	50	12	27.2	22.8	1.26
2*	23	11	50	12	19.8	14.3	0.92
2*	18	2	100	12	38.7	33.8	1.97
2*	34	9	100	12	42.3	34.3	0.83
2*	32	7	150	12	43.9	38.3	1.64
2*	36	11	150	12	45.3	38.0	1.46
2†	37	18	50	12	34.3	24.0	1.02
2†	42	24	50	12	35.0	23.9	1.07
2†	29	14	100	12	60.0	51.2	2.66
2†	42	24	100	12	49.0	38.0	1.32
2†	41	23	150	12	78.5	67.5	2.22
2†	42	24	150	12	66.7	55.7	1.69

* undried. † dried.

Column 1: serial number; the series are designated in order of receipt.
Column 2: plate number; the plates are designated in order of use.
Column 3: time in days which elapsed from arrival of the plates in Copenhagen till their actual use.
Column 4: exposure time in minutes. Multiple exposure plates have been exposed for varying lengths of time in different areas of the same plate (cf. p. 15).
Column 5: number of fields counted within the image.
Column 6: mean number of tracks counted per $(100)^2\mu^2$ of the image; this figure equals the mean of the track counts actually obtained, divided by the ratio: field size in sq. microns/$(100)^2$ sq. microns.

$$\text{Mean of } X_i = \overline{X}_i = \frac{\Sigma^n X_i}{n}.$$

Column 7: net mean track count per $(100)^2\mu^2$; this figure equals the mean track count per $(100)^2$ sq. microns of the image minus mean track count per $(100)^2$ sq. microns from background areas of the same plate.
Column 8: standard error of the net mean track count per $(100)^2\mu^2$. Standard error $= s$ (of net mean count per $(100)^2$ sq. microns)

$$\text{S. E.} = \sqrt{s^2_{\substack{\text{mean exp. count}\\ \text{per } (100)^2\mu^2}} + s^2_{\substack{\text{mean background count}\\ \text{per } (100)^2\mu^2}}}$$

$$\text{Variance of the mean of } X_i = s^2_{\overline{X}_i} = \frac{s^2_{X_i}}{n}.$$

TABLE 2.

Counting Data from Plates of Series 2–5, as depicted in Fig. 1.

1 Series	2 Plate	3 Days between arrival and exposure	4 Exposure time in min.	5 Number of exposed fields counted	6 Mean exposed count per $(100)^2\ \mu^2$	7 Net mean count per $(100)^2\ \mu^2$	8 S. E. of net mean count per $(100)^2\ \mu^2$
2†	37	18	50	12	34.3	24.0	1.02
2†	42	24	50	12	35.0	23.9	1.07
2†	29	14	100	12	60.0	51.2	2.66
2†	42	24	100	12	49.0	38.0	1.32
2†	41	23	150	12	78.5	67.5	2.22
2†	42	24	150	12	66.7	55.7	1.69
3	A 9	3	50	10	15.6	12.3	1.22
3	A 7	2	100	10	33.6	30.1	1.09
3	A 11	3	100	10	36.1	30.3	1.52
3	A 10	3	150	10	40.6	37.9	1.82
3	B 1	15	50	10	17.3	14.1	1.92
3	B 5	43	50	10	19.8	16.2	1.24
3	B 8	59	50	10	21.4	15.5	0.85
3	B 3	22	100	10	42.6	39.0	1.85
3	B 6	43	100	10	47.0	40.5	1.46
3	B 2	16	150	10	60.0	55.4	2.00
3	C 2	4	50	10	16.1	12.3	0.87
3	C 6	9	50	10	19.5	16.8	0.90
3	C 8	10	50	10	16.4	13.9	1.24
3	C 9	15	50	10	16.5	14.6	0.70
3	C 12	37	50	10	18.2	14.9	1.31
3	C 2	4	100	9	33.9	30.1	1.45
3	C 8	10	100	10	29.0	26.5	1.85
3	C 11	22	100	10	29.0	27.4	1.10
3	C 6	9	150	10	52.4	49.7	2.27
3	C 10	16	150	10	53.8	51.1	1.82
4	5	8	50	10	16.5	13.0	0.96
4	6	9	50	10	14.8	12.4	0.75
4	5	8	100	10	29.6	26.0	2.13
4	6	9	100	10	30.0	27.6	1.69
5	5	4	50	10	15.9	13.3	1.29
5	8	5	50	10	14.0	11.9	1.44
5	6	5	50	10	17.1	15.1	0.85
5	6	5	100	10	26.5	24.5	1.86

† dried.

In computing the standard error of the net mean track count for plates from batches where there is a tendency for contiguous fields to yield similar track counts, the variance of the mean exposed count and of the mean background count was estimated from the variance of the means of groups of contiguous fields (exposed or background). The standard errors are given here for descriptive purposes, only. In view of suggested differences between different plates of a batch and between different batches, they are not considered to be valid estimates of the error of the respective mean track counts.

C and D were students who did not participate in the research work proper, but were trained for the special task of track counting. The purpose of their participation in the counting was for the present authors to gain an impression of the difficulties inherent in this work, especially because it requires personal judgement and a certain consistency of interpretation.

Reproducibility of counting results.

In order to estimate what proportion of the variability observed in the field counts of one investigator is attributable to uncertainty of evaluating a given track pattern, investigator B recounted all ten selected fields on each of four different images (series 3) after a lapse of several months. This investigator also recounted a single field from each of two exposed areas five times on five different days to provide an additional check on her counting variability.

The variance of the two track counts of the same fields was computed for each of the ten fields from each of the four images recounted, and the mean of these ten variances from a given image was calculated. The results provide estimates of investigator B's average variation in evaluating the track pattern at the exposure levels 50 — 100 — 150 minutes, respectively. The average variances found represent, at the 50 min. exposure level, 4 $^0/_0$ of the variance of counts of different fields, 6 $^0/_0$ at the 100 min. exposure level, and 10 $^0/_0$ at the 150 min. exposure level. Thus, the variance of recounting given track patterns increases with increasing number of tracks per field, not only as an absolute value, but also as a percentage of the variance of different fields.

The estimated standard deviation of recounts of fields, the square root of the average variance discussed above, appears to be a fairly constant percentage of mean track count: 4.5 $^0/_0$ on the 50 min. exposure level, 4.4 $^0/_0$ at the 100 min. exposure level, and 3.5 $^0/_0$ at the 150 min. exposure level.

The track counts of the single fields counted on five different days yield variances smaller than any of the average variances of two counts of the same field reported above. Thus, these limited observations provide no evidence of a day to day variance greater than that included in the estimates given above.

No recounts by the other three investigators are available. Since the variance of the field counts is of the same order of magnitude for all four investigators, it is unlikely that the recount variation of the other investigators (especially A) is vastly different from that of B.

The two most experienced investigators, A and B, showed, on the whole, very good agreement. The data give little evidence of any overall tendency of one of these investigators to count, on the average, higher or lower than the other.[1] For seven images on plates of series 3, on each of which the same ten fields were counted by both A and B, the difference between the mean track count of A and of B, in per cent of mean track count, averages less than 4 $^0/_0$. There is no tendency for the percentage difference to increase with increasing track count. This close agreement between mean counts of A and B is confirmed by the data from the other series.

For the eighteen sets of paired counts, the standard deviation of the difference between the counts of the same field by A and B ranges from 2 $^0/_0$ to 12 $^0/_0$ of the mean track count. On the average, the standard deviation of the differences between A and B is only about 50 $^0/_0$ higher than the standard deviation of the differences between two counts of the same field by investigator B.[2]

To *summarize:* for an experienced investigator, the variation in repeated evaluations of the same track pattern at different

[1] Heterogeneity of variance was indicated by the data even within a single exposure level, so the data from different images could not be pooled. For plates of the first two series, the fields counted by both A and B vary in number from image to image. For seven images from series–3 plates, the number of fields from each image counted both by A and B is ten. The mean of the three mean differences was tested by the statistic t. For the three paired means at 50 min., t is $+ 0.4$, at 100 min., t is $- 1.3$. The single mean difference at 150 min. is positive.

[2] Comparison of the counts obtained by B and D on the first group of plates counted by D (largely series 2) reveals a pronounced and consistent tendency of D to count (on the average 6 $^0/_0$) lower than B. This tendency is not evidenced on plates counted subsequently. As investigator D gained experience, he evaluated the track pattern differently so as to obtain higher counts. Investigator D never attained as good agreement with B as A exhibited during the entire course of the study.

Investigator C counted only plates of series 1. Counts of three investigators, A, B, and C, are available for nine plates. The mean counts of C seldom show good agreement with those of A and B. Investigator C's mean counts differ on the average from those of A and B by two to three times as much as their mean counts differ from each other.

No comparative data are available between investigators C and D.

times appears to be small. The variance of recounting represents an increasing proportion of the investigator's variation in counts of different track patterns from an image as the average number of tracks per field increases; but even at 150 min. exposure (ca. 80 tracks per field), the variance of recounts accounts for only 10 % of the variance of counts of different fields. The standard deviation of recounts—which is the pertinent statistic in terms of error—shows, however, no tendency to increase with increasing track count, and represents only about 4 % of the track count.

Two of the four investigators showed on the whole very good agreement on counts of the same fields. For the great majority of images, there is no consistent tendency for one of these investigators to count on the average higher or lower than the other, and their counts of the same field differ by only about 1.5 times as much as do two counts of the field by the same investigator.

Homogeneity of the plates.

When a number of fields within the image from a standard source are counted, the scattering of the results around a mean value is a function of (A) the statistical fluctuation in the number of disintegrations actually taking place per time unit, (B) the uncertainty of evaluation as discussed above, and (C) the error in the reproduction of the track pattern, which includes the effect of inhomogeneity within a plate. If fields from images on different plates are considered, lack of homogeneity from plate to plate within the same batch, and also variations in sensitivity from batch to batch would introduce further variability. Homogeneity in this sense is synonymous with uniformity of sensitivity. If the photographic emulsions are homogeneous, any unit area at one end of a plate will be as sensitive to incident radiation as a corresponding area at the other end of the same plate, or on a different plate from the same batch, or from another batch.

(i) *Homogeneity within a plate.*

Striking inhomogeneity within one and the same plate was observed on plates of series 1 and on some plates from later series. On five of the nine plates of series 1, a large number of exposed and background fields was counted in groups of four contiguous fields about a common center (the landmark, cf. p. 5). Scanning of the individual track counts of a given investigator from a given image revealed a striking tendency for contiguous fields to yield similar track counts, which was confirmed by analysis of variance. This phenomenon appears consistently in counts from images of this series and, to some extent, in the background counts as well.

In series 2—5, the exposed fields were counted in groups of two contiguous fields, the background fields usually in groups of four.

The majority of the plates of series 2 show no tendency for contiguous fields to yield similar track counts. However, in this series and also in series 4 and 5, a few sets of counts evidence this grouping effect to a "significant" degree.[1] By contrast, the extensive data from series 3 give no indication of the existence of such a grouping effect. The corresponding background counts suggest that contiguous fields may be more similar than fields in widely separated areas of the plate. The data, however, are inconclusive.

On the basis of the actual counting experience, the investigators maintain that subjective factors cannot explain this tendency for contiguous fields to yield similar track counts. During the counting of plates of series 1, the only batch in which the phenomenon is consistently present, areas as large as a quarter of a field were frequently observed to be completely devoid of tracks. In later series, this phenomenon was observed in rare cases, only. Wherever this grouping effect exists, a lack of homogeneity within very small areas of the plate is suggested.

In the light of these findings, it seems advisable to count separately located fields rather than groups of contiguous fields in order to obtain a maximum amount of information from a given amount of counting labor. Therefore, in series 2—5, the

[1] For four of the five images from series 1, the counts of investigator B yield variance ratios (F) which are significant at the 5 $^0/_0$ level of confidence or better. The same is true of two of the five sets of background counts. In addition, three out of twenty-two sets from series 2 (two of which occur on the same plate), one out of six sets from series 4, and one out of seven sets from series 5 yield F ratios which are significant at the 5 $^0/_0$ level of confidence. For all of these series, statistical analyses of the counting data of the other investigators give similar results.

exposed fields were counted in groups of two contiguous fields, the backgrounds usually in groups of four.

(ii) *Homogeneity of plates from the same batch.*

In order to study the homogeneity—or uniformity of sensitivity—from plate to plate of the same batch, the net mean track counts from all images of the same exposure level were compared.

The data from series 2 (dried and undried groups considered separately) suggest that within this batch some plates, or areas of them, differ from others in such a way that they record as tracks differing percentages of the radiation received.[1]

Because of variations in the handling of plates of series 3 and other considerations, statistical analysis of this batch of plates is not informative.

Series 4 and 5 comprise only a small number of plates and no evidence of the existence of differences between plates within these batches was found.

(iii) *Sensitivity from batch to batch.*

Figure 1 presents the net mean track counts of investigator B from all images studied, except those from plates of series 1[2] and series 2 undried. The means from plates of series 2 dried are consistently higher at every exposure level than the means from plates of series 3, 4, and 5.

This phenomenon is confirmed by the data from the other investigator (D).

This finding indicates that different batches of plates may differ appreciably in average sensitivity, and it seems not justified to assume that NTB-2 plates from different batches will yield the same number of tracks per field for a given exposure to the same source of radiation.

[1] Counts from images on two different plates are available at the three exposure levels in each group (dried and undried). In each case, the mean difference was tested by the statistic *t*. For investigator B, three of the six mean differences (at 50 min. in the undried group, at 100 min. and 150 min. in the dried group) are significant at the 2 % level of confidence. Counting data from the other investigator who counted this series yield almost identical results. The possibility of a somewhat reduced reliability of this test when applied to these data must be considered.

[2] Cf. footnote on p. 5.

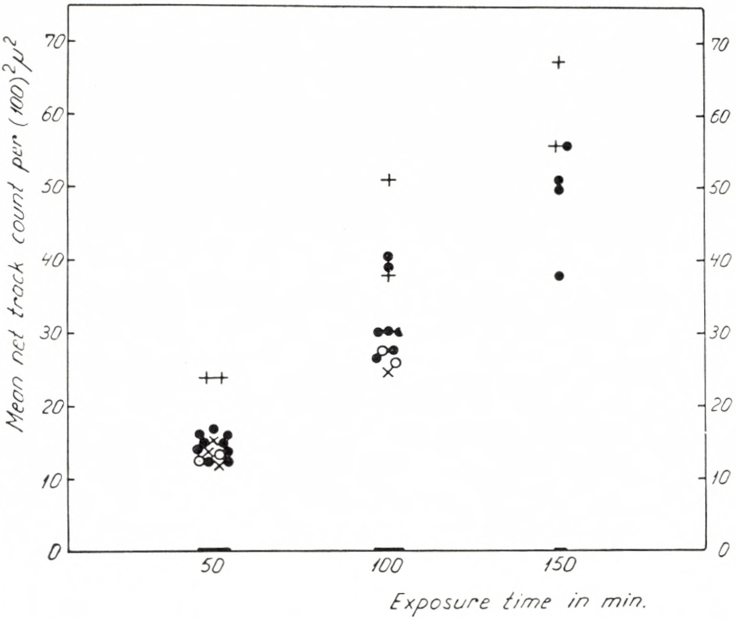

Fig. 1. Mean net track count per field ($(100)^2 \mu^2$) versa exposure time in minutes. + series 2; ● series 3; ○ series 4; × series 5.

Investigation of saturation phenomena (*H* and *D* relation).

The dependence of track count on exposure time was studied applying the exposure times 50 min., 100 min., and 150 min. The experiments were made in two different ways, namely, either by exposing three plates from the same batch, or by exposing different areas of the same plate, for increasing periods of time. The limitation to three different exposure times was necessary because counting is too laborious to allow of a much greater number of images to be evaluated.

The results are illustrated in Fig. 1.

When each batch of plates was considered separately, it was found that the means of the three exposure levels deviate somewhat from the 1:2:3 relationship, and that deviations occur in both directions.

The largest deviation from the 1:2:3 relationship is evidenced by the data from the undried plates of series 2, and is in the direction indicating the presence of a saturation phenomenon,

i. e., the net mean track count increases less than would be expected with increasing exposure time. For the remaining five groups of plates, two exhibit deviation in the direction of saturation, three show deviation of comparable magnitude in the opposite direction.

Three of the seven single plates on which exposures at two or three different levels were made (so-called multiple exposure plates) show deviations from the 1:2:3 relationship almost as large as the largest observed in the means of the batches. However, while two of these plates exhibit substantial deviation in the direction of saturation, the deviation of the third is of comparable magnitude in the opposite direction.

Thus, the data as a whole give little evidence that saturation has been reached. In view of the findings as reported in the previous sections, the sizable deviations from the 1:2:3 relationship observed within some single plates may well be due to inhomogeneity within the plate.

Efficiency of the plates.

When a source of beta particles is brought in contact with a photographic emulsion, only slightly less than 50 $^0/_0$ of the disintegrations taking place in the source will be "seen" by the emulsion due to geometric conditions. If the source is thick as compared with the range of the beta particles, an additional correction for self-absorption must be applied. In the present case, the source was an "infinitely thick layer" of C-14 labeled polystyrene and therefore, as is well known, only about $^1/_5$ of the disintegrations in the direction of the detector will reach the emulsion. Taking the correction for geometry and for self-absorption into account, we can expect at the most $^1/_{10}$ of the disintegrations taking place in the foil to be registered by the photographic plate.

Some additional loss may occur due to the interspace (of about 1—2 microns) between source and emulsion despite the fact that these surfaces are pressed together, and it is also possible that particles traveling in the emulsion very close to the surface may not be registered as tracks.

We thus arrive at a figure for the number of tracks to be expected per unit field (100×100 square microns) of the order of 10 % (or somewhat less) of the disintegrations taking place in a piece of the foil of the same surface dimension and a thickness corresponding to the range of the C-14 beta particles.

The standard used contains 0.6 microcuries of C-14 per sq. cm., and, thus, 650 disintegrations take place in the course of 50 min. in 100×100 sq. microns of the foil. The order of magnitude of the track count should therefore be 65 tracks per unit field after 50 min. exposure (or somewhat less).

The actual finding, as illustrated in Fig. 1, is lower than the above estimate by a factor of about 4. The discrepancy may be due to the fact that the C-14 content of the standard foil is not too well-defined. It is also possible—as mentioned above—that the contact between source and emulsion was poorer than anticipated. As pointed out by Pelc et. al.[2], this distance is of great importance. Finally, the possibility does exist that the NTB-2 emulsion used did not register all incident particles as tracks, in other words, the efficiency of the emulsion when applied in the manner described here is not one hundred percent. It is worth emphasizing that, in this sense, efficiency is not synonymous with sensitivity, a concept which has been defined and is being used differently.[4]

In experiments carried out with the same standard source placed on an Ilford G-5 emulsion under otherwise identical conditions, the G-5 emulsion registered on the average 45 tracks per unit field after 50 min. exposure, i. e. about 2.5 times more than did the NTB-2 plate.

Effect of storage conditions.

The effect of storage conditions, i. e., temperature, shielding, and humidity, on the efficiency of the plates was studied. It is generally assumed that the lifetime of the plates is prolonged by storage in the cold, that high humidity promotes latent image fading, and that storage in a lead (iron) shield of 5—10 cm thickness will reduce the accumulation of background, especially in the case of emulsions which register electrons from cosmic radiation as tracks. Some of the experiments were extended over a period of $1^1/_2$ months, and not only the net track count, but also the increase in the background count was considered to be of interest.

The plates of series 3 (a total of three dozen) were stored in different ways in order to study the effect of temperature and

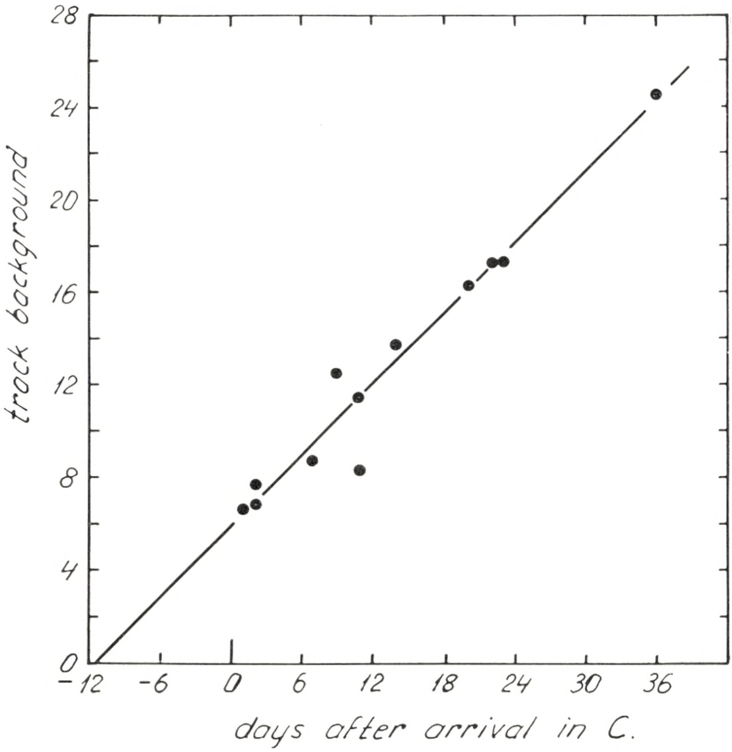

Fig. 2. Increase in track background count per field ($(100)^2\mu^2$) versa time, expressed in days after arrival of the plates in Copenhagen. The day of manufacture is not stated by the producer, shipment took 5 or 6 days.

shielding over an extended period on the properties of the plates. The plates of group A were used within three days after arrival, those of group B were stored at + 5° C in the refrigerator, some for as long as 59 days. The plates of group C were stored at room temperature (20°—22° C) inside a 20 cm iron shield, some for as long as 37 days. Comparison of the data from these three groups reveals no tendency for efficiency to decline with storage under either condition.

At every exposure level, all plates of group B yield higher net mean track counts than any plate of group A. The mean of all group-B plates is higher at every exposure level than the corresponding mean of group-C plates. Comparison of group-A plates with group-C plates yields no consistent difference. The only large difference observed, that between the 150 min. means of the two groups, may be due to differences between plates, as found in other batches.

Fig. 2 shows the increase in background count with time on plates of series 2, which were not shielded during storage. The plates of group C, series 3, stored in a massive iron shield, show no increase in mean background count with time. The background of series-3 B plates stored in the refrigerator, but unshielded remained lower than did that of series 2, although storage was extended over 59 days.

Drying with silica gel prior to exposure was first used with the plates of series 2. At each exposure level, the net mean track counts of dried plates of series 2 are higher than those of undried plates. Application of the theory of combinations reveals that the probability of obtaining such results by chance, i. e., if the drying process actually had no effect on the mean track count, is 1 in 108. Therefore, it seems reasonable to conclude that careful drying of the emulsion prior to exposure does increase the efficiency of the plates of at least some factory batches.

Discussion.

It has been the aim of the present study to investigate the applicability of NTB-2 plates to quantitative β-track autoradiography, for example in connection with tracer studies on biological specimens.

For *practical purposes*, tissue sections, 5—10 microns thick, will be floated on the plates, for example, in the manner described by Evans[3], and the autoradiogram be observed in the emulsion under the tissue. When using this technique, it must be kept in mind that the sensitivity of the plates is affected by changes in humidity and, therefore, the plates must be dried carefully after having been immersed in water. When most of the moisture has been removed by drying with a fan, the plates must be dried more thoroughly, e. g., with silica gel. After too rigorous drying, the emulsion begins to peel off, and it will largely be a matter of trial and error to find optimum drying conditions. As long as the humidity of the plates is controled, storage conditions are not critical. The use of a shield is advantageous because accumulation of the track background thereby is suppressed.

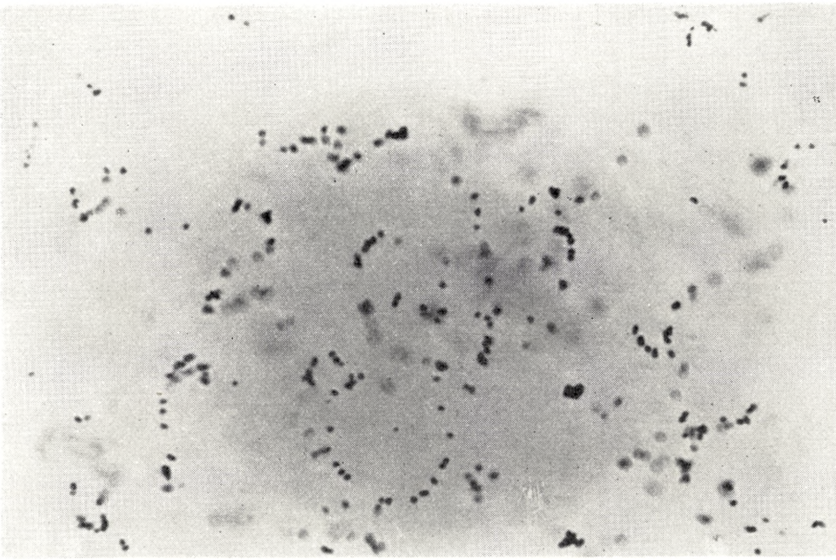

Fig. 3. Photomicrograph of a C-14 beta track pattern in NTB-2 emulsion. Depth of focus about 3 microns.

Exposure time must be such that the number of tracks does not exceed c. 100 per 10^4 sq. microns. At a higher level, counting becomes too difficult. Up to this track density, the number of tracks per field versa exposure time appears to be a linear relation.

Presupposing standardized handling of the emulsion, *the reliability of the counting results* depends on the experience of the investigator and on the properties of the plates.

The investigators must train themselves and establish well-defined criteria on which the interpretation of the track pattern is based. Track counting is not an easy technique, especially because it requires the investigator's full attention and consistency of evaluation. Fatigue is a source of error not to be underestimated. Investigators C and D, participating in this study, were not particularly interested in perfecting themselves or in improving the method. This is probably part of the reason why their counting results were less reproducible than those of investigators A and B.

In the present study, the standard deviation of recounts of fields was about 4 % of the mean track count. The average difference between the mean track counts obtained by the two main investigators counting the same ten fields of a given image was about 4 %. An estimate of the standard deviation of single net track counts of one investigator (B), i. e., the count from one exposed field less the count from one background field as calculated from the average of the variances within individual plates of a series is given in Table 3.

TABLE 3. Estimated Standard Deviation of Single Net Counts (% of net mean track count).

	50 min.	100 min.	150 min.
series 1	38 %	20 %	18 %
series 2, dried	20 %	12 %	11 %
series 3, 4, and 5	26 %	16 %	13 %

The lack of homogeneity of some of the plates is a serious problem. If the mean track count of fields from a given image is used to estimate the amount of radiation received—as in comparisons between different regions of one image, or between images obtained from unknown sources of radiation—the error calculated from variance of track counts within the exposed areas as mentioned above may seriously overestimate the reliability of the mean count obtained. The differences in sensitivity which were found to exist between different areas of some plates and between plates of some batches are not included in the errors given in the above table. They contribute additionally to the uncertainty of the final results. Differences observed between plates are of such magnitude as to make the error of net track counts from different plates 1.5 to 2 times the error for fields from the same plate. The use of only one plate for all images—even where feasible—cannot be assumed to eliminate this additional source of error. A further decrease in reliability of the results may arise if images made on plates from different batches are compared.

In practice, this means that a comparison of net track counts per field under different regions of a tissue section can only lead to reasonably accurate results if the regions to be compared are so large that a suitable number of scattered fields can be counted in each region. In view of the inhomogeneity observed within very small areas of some plates, it is advantageous to count separately located fields wherever possible. Comparisons based on single field counts will require the study of serial sections, preferably mounted on one and the same plate.

Very little quantitative information on the *resolution* in a beta track autoradiogram can be obtained from standard exposures with a source of infinitely thick layer. In a medium of density 1 (polystyrene, paraffin, tissue) the range of C-14 betas is about 30 microns, in emulsion (density 4) it is correspondingly less, and in air it is about 3×10^4 microns. When a thick standard source is pressed against the emulsion and the interspace is kept small, the beginning of the tracks will not be too distant from the particles' "points of origin" in the source. With thin tissue sections placed on the emulsion, particles emitted in the direction away from the emulsion can easily reach a medium of low density (air) and can be scattered back into the emulsion, forming a track at considerable distance from their points of origin. It is very tempting, but definitely not justified, to project the starting point of a track as seen in the microscope vertically into the superimposed tissue, assuming that the track originates from this point in the tissue. Systematic investigations into this problem are in progress. The authors' estimate of the resolution obtained when tissue sections containing a C-14 labeled compound are mounted on NTB-2 plates is about 15 microns.

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Present address: 3500 Woodridge Ave., Wheaton, Md.

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Department of Biological Isotope Research,
Zoophysiological Laboratory,
University of Copenhagen.

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