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# Sensitivity Enhancement of Ninhydrin-Treated Latent Fingerprints by Enzymes and Metal Salts

**REFERENCE:** Everse, E. and Menzel, E. R., **"Sensitivity Enhancement of Ninhydrin-Treated Latent Fingerprints by Enzymes and Metal Salts,"** *Journal of Forensic Sciences*, JFSCA, Vol. 31, No. 2, April 1986, pp. 446-454.

**ABSTRACT:** Lyophylized Sigma Type III trypsin has been applied to latent prints two weeks to two months old. This trypsin preparation eliminates the background problems that had been encountered with old prints in a previous study. Zinc chloride treatment of latent prints previously exposed to ninhydrin enhances their detectability upon laser examination. However, it has been reported that the zinc chloride reaction occasionally fails to occur. Accordingly, we have investigated the optimization of this reaction. We find that high humidity and elevated temperature, particularly the former, are needed. Cadmium nitrate, although it produces weaker fluorescence than zinc chloride, may at times be useful. Reaction conditions are much the same as those for zinc chloride.

**KEYWORDS:** criminalistics, fingerprints, ninhydrin, enzymes, trypsin, zinc chloride, cadmium nitrate, lasers

Ninhydrin has become the universal reagent for the chemical development of latent fingerprints on porous surfaces such as paper. But although ninhydrin is a sensitive reagent for the detection of amino acids, its usefulness as a method for the development of latent fingerprints is limited by the availability of amino acids in the prints. Three factors play a role in the total amount of amino acids present in fingerprints: (1) the total amount of material deposited in the print, (2) whether the individual is a strong or weak excretor of amino acids in palmar perspiration, and (3) the age of the print.

In recent years, with the increasing use of laser technology by law enforcement agencies, the ultrasensitive latent fingerprint detection method of treating the ninhydrin-developed surface with zinc chloride is being utilized in an increasing number of forensic science laboratories. Under the blue-green light of the argon laser the zinc salt of the product of the ninhydrin/amino acid reaction fluoresces strongly [*1*]. The ninhydrin/zinc chloride fluorescence method will in many instances develop prints with good detail where none are visible on the ninhydrin-developed article under room light. Yet this method, although far more sensitive than the use of ninhydrin alone, is still limited by the availability of amino acids.

Another approach has been taken to improve the enhancement of latent print detection by the ninhydrin method. Recently, Menzel and coworkers reported that an increase in ninhydrin reactivity can be achieved by the use of the pancreatic enzyme trypsin [*2*]. The rationale for this procedure is that it increases the amount of ninhydrin-reactive material in the print. The en-

Received for publication 22 March 1985; revised manuscript received 1 July 1985; accepted for publication 3 July 1985.

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zyme trypsin catalyzes the cleavage of the proteins that are present in palmar perspiration. The results presented in the initial study establish the use of trypsin as a valuable addition to the ninhydrin enhancement of latent fingerprints.

There have been some inconsistent results with the use of trypsin, however. The major problems have been either a high background or the loss of detail during incubation. In particular, difficulties were encountered in treatment of old prints [*2*]. This article reports the results of a study designed to understand the mode of enhancement to establish the conditions for optimal enhancement and determine the applicability of trypsin to the enhancement of aged prints. Several enzyme preparations and various incubation times and temperatures are compared.

It has been reported repeatedly by law enforcement laser users that the zinc chloride ($ZnCl_2$) reaction following ninhydrin is at times either very slow or fails to materialize altogether. To remedy this occasional problem, we have made an optimization study of the ninhydrin/$ZnCl_2$ treatment as a function of reaction conditions. The ninhydrin/$ZnCl_2$ treatment is best suited to use of the 488-nm Ar-laser line (absorption maximum at 490 nm). With this illumination, however, some papers exhibit strong background [*3*]. Substitution of cadmium nitrate [$Cd(NO_3)_2$] shifts the absorption maximum to about 510 nm, so that 510-nm Cu-vapor or 514.5-nm Ar-laser illumination can be used, and this may reduce background problems even if the ninhydrin/$Cd(NO_3)_2$ fluorescence efficiency is not as high as that of ninhydrin/$ZnCl_2$. Cadmium nitrate may also be preferable if frequency-doubled Nd:YAG laser excitation is used. Thus, we have studied cadmium nitrate as a complement to zinc chloride.

## Materials and Methods

### *Sources*

Trypsin was obtained from Sigma Chemical Co., St. Louis, MO, and from Difco Products, Detroit, MI. Ninhydrin was obtained from Sigma Chemical Co., St. Louis, MO. All other reagents were obtained from Fisher Scientific, Houston, TX.

### *Ninhydrin*

A stock solution of this reagent was prepared by dissolving 48 g of ninhydrin in 100 mL of methanol. Before use, the ninhydrin solution was diluted with freon (1,1,2, trichloro-1,2,2 trifluroethane) in the proportion of 20% methanol solution to 80% freon by volume.

### *Zinc Chloride or Cadmium Nitrate*

A stock solution of the reagent was prepared by dissolving 37 g of zinc chloride in 100 mL of methanol. Before use, a working solution was prepared in the proportion of 20% methanol solution to 80% freon by volume. The working solution must be shaken vigorously immediately before use to form an emulsion. Cadmium nitrate solutions were prepared with the same concentration of the salt.

### *Application of Trypsin*

Trypsin was kept stored in a dessicator either in the refrigerator or freezer according to manufacturer's directions. It is important that the enzyme be kept dry; moisture promotes the loss of enzymatic activity by self-digestion. To prevent condensation in the trypsin preparation, the trypsin bottle was allowed to warm to room temperature before opening. Trypsin was applied either by sprinkling on one edge of an article and then tapping gently to cause an even distribution over the surface or by gentle application with a brush. Magnetic powder also worked well. However, the reuse of such powder increases the risk that the enzyme will become moist and

lose its activity. The article was then set in a moist chamber at 37°C and allowed to incubate for a specified time. After incubation, the trypsin-treated surface was allowed to dry and the trypsin was removed by blowing the powder off with a stream of nitrogen. It was necessary to remove the remaining trypsin to prevent high background coloration.

### *Print Samples*

Fingerprints were deposited on various paper types. Prints were aged in ambient room conditions. Prints from zero to two days age are called fresh prints; those from fourteen to sixty days are called aged prints. Prints were cut in half. One half was used for the treatment to be tested, and the other half served as control.

## Trypsin for Enhancement of Fingerprint Development

### *Comparison of Various Trypsins and Reaction Conditions*

Sample half-prints on four different paper surfaces were dusted with one of three trypsin preparations varying in particle size and purity. The other corresponding half-prints were not treated with enzyme and served as a control. Both enzyme-treated and control samples were incubated for an hour at 36°C in a humid atmosphere. The ninhydrin reaction is known to be affected by temperature and moisture; thus, it is essential to subject the controls to the same ambient conditions as the enzyme-treated samples. After the incubation all samples were sprayed with ninhydrin. When the ninhydrin development was complete the samples were compared. The various enzymes yielded different degrees of enhancement depending on the porosity of the paper, but in all cases the enzyme treated side had far greater ninhydrin development. Two Difco trypsins, Trypsin 1:250 (0152-13-1) and Bacto Trypsin (0153-59), were examined. Both preparations, which were fine powders, provided strong enhancements that were nearly obscured by a high background on the more fibrous rough papers. The Sigma Type III trypsin provided a lesser enhancement of the ninhydrin color with no background problems. Sigma Type III trypsin had the greatest specific activity of the enzymes tested but did not produce the greatest ninhydrin color. These results suggest that factors such as particle size also play a role in the degree of enhancement of latent prints with trypsin.

To test this possibility, it was necessary to decrease the particle size of the Sigma Type III trypsin. This was done by dissolving 250 mg of the large fluffy particle trypsin in 10 mL of water that was slightly acidified with acetic acid. This solution was frozen to the walls of a 100-mL beaker and lyophylized until dry [*4*]. The result of this treatment was a fine white powder. This trypsin preparation was compared with the Difco preparations and the fluffy Sigma trypsin for latent print enhancement. The finely powdered, lyophylized Sigma Type III trypsin gave the most enhancement with no background difficulties. Thus, this trypsin preparation was used in all subsequent experiments.

To ascertain the optimum time for incubation of latent prints with trypsin, the following experiment was performed on fresh prints. Half-print samples were dusted with trypsin and incubated at 37°C, 90% relative humidity, for various time intervals. The corresponding control half-prints were treated in the same manner but had no enzyme applied. At the end of the incubation period excess trypsin was removed with a blast of nitrogen through a small nozzle. Then, all the samples were sprayed with the ninhydrin reagent and allowed to develop in the incubator at 37°C, 90% relative humidity. The trypsin incubation time intervals taken were 0, 5, 10, 15, 20, 25, 30, 35, 45, and 60 min. The trypsin-treated samples showed increasing purple color. After 30 min the increase in color development slowed and reached a maximum at 45-min incubation. The untreated controls had less ninhydrin development than any of the enzyme treated samples, including the no incubation sample.

To evaluate the contribution of trypsin as an enzyme in comparison with trypsin as a self-

activating dusting powder, print samples of from two weeks to two months age were used because aged prints do not dust well. The left half of a set of prints was dusted with Sigma trypsin and was allowed to incubate for 40 min at 36°C, 90% relative humidity. The corresponding control right half was dusted with trypsin and incubated at room temperature in a dry box for 4 h. Figure 1 shows the result. Another set of prints was dusted with trypsin on one side and set to incubate 40 min at 36°C, 90% relative humidity. The corresponding half was not treated with trypsin but was incubated in the same manner. Figure 2*a* shows the result. A third set of prints had half dusted with trypsin; the corresponding half received no enzyme. Both halves were incubated in a dry box for 3 h at room temperature. Figure 2*b* shows the result. As an ancillary experiment, incubation in a moist environment was examined as a function of temperature between about 15 and 37°C. As expected for enzymatic action, the reaction rate increased with temperature.

An experiment similar to that above was performed on aged prints which had first been treated with ninhydrin and then cut in half. It was necessary to incubate for 2 h at 36°C, 90% relative humidity, to obtain maximum enhancement after trypsin application. The corre-

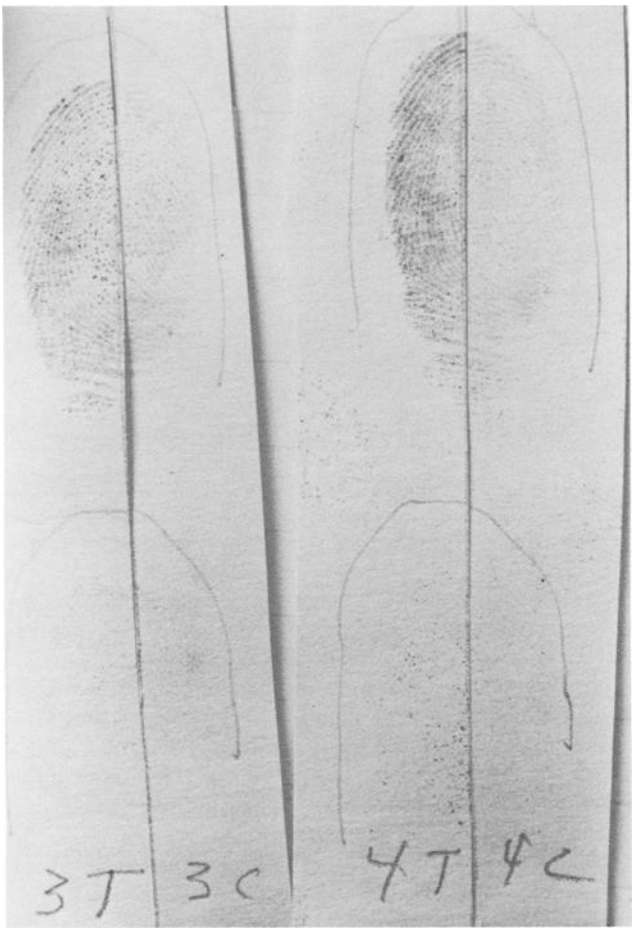


FIG. 1—*Fingerprint samples treated with trypsin and then ninhydrin. The left halves of the prints were incubated at 37°C, 90% RH for 40 min before ninhydrin treatment, the right halves were incubated at room temperature in a dry box for 4 h before ninhydrin treatment.*

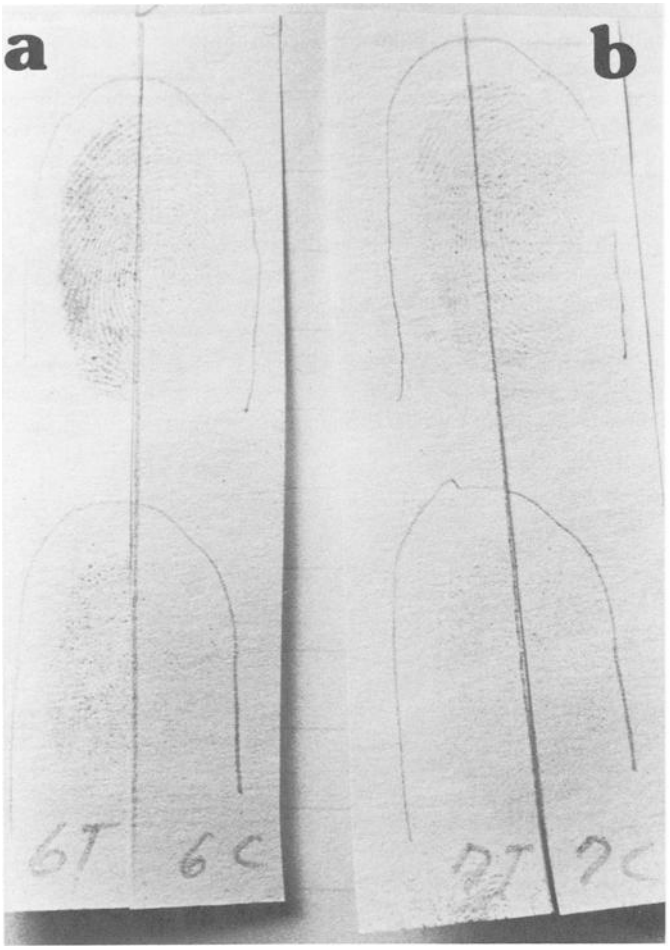


FIG. 2—*Fingerprint samples with left halves treated with trypsin and then ninhydrin, and with right halves treated with ninhydrin only. Before ninhydrin treatment, both halves of* 2a *were incubated at 37°C, 90% RH for 40 min, both halves of* 2b *were incubated at room temperature in a dry box for 3 h.*

sponding controls were stored for 18 h in a dry box at room temperature. The trypsin-treated side that was incubated in a humid atmosphere at 36°C was enhanced over the no enzyme control as well as over the trypsin-treated samples that were stored in the dry box. The samples stored in the dry box showed the same development as the no enzyme controls.

The degree of enhancement of trypsin-treated samples incubated at 36°C, 90% relative humidity, was considerably greater if the sample was treated with ninhydrin after incubation with the enzyme rather than before. This is readily understood in terms of fingerprint residue penetration into the paper as a result of the (solution) ninhydrin treatment, hence reduced contact between enzyme and protein molecules. An experiment was performed to assess whether or not trypsin could be used to enhance prints that had been treated with zinc chloride. A series of fingerprints that had been developed with ninhydrin and then treated with zinc chloride were cut in half. One side was treated with trypsin, the other side served as a no enzyme control. After incubation for 40 min at 37°C, 90% relative humidity, both sides had lost detail. The trypsin-treated side was noticeably wet, and the excess trypsin could not be removed with a blast of nitrogen—after it had dried.

## Discussion

In a previous article this laboratory reported that treatment of latent fingerprints with trypsin, followed by development with ninhydrin and zinc chloride, causes a dramatic enhancement in the fluorescence and therefore in the detectability of fingerprints. Fingerprints contain substantial amounts of proteins in addition to free amino acids; in comparison to free amino acids, however, proteins react weakly or not at all with ninhydrin. The rationale for the trypsin treatment was that an enzymatic separation of the fingerprint proteins into free amino acids would increase the amount of free amino acids in the prints and cause a more intense ninhydrin staining. As reported previously, this was indeed observed in our experiments.

However, several questions arose concerning the mechanism by which trypsin causes this enhancement in ninhydrin color. First, because the amount of trypsin adhering to a fingerprint is possibly many times the amount of the fingerprint protein present, and because trypsin does react with ninhydrin (albeit much more weakly than the reaction of free amino acids with ninhydrin), it is conceivable that the observed increase in ninhydrin color is primarily due to the presence of the trypsin itself rather than to any additional free amino acids formed by the trypsin. In other words, it is conceivable that trypsin acts simply as a dusting powder that reacts with ninhydrin.

The results presented here for aged prints invalidate this possibility, since the enhancement by trypsin was shown to be a time-dependent, moisture-dependent, and temperature-dependent phenomenon. Incubation of trypsin-treated prints for various lengths of time at 37°C produced an increased response, reaching a maximum at an incubation time of about 45 min. This is exactly what one expects to observe if trypsin functions as an enzyme (producing free amino acids), but these observations are inconsistent with the concept that trypsin serves solely as a dusting powder. Clearly, the trypsin used in our earlier study [*2*] contained impurities that react with ninhydrin, and this caused the strong background and generally negligible enhancement observed for old prints.

A second possibility is that the increase in ninhydrin color is the result of the autolysis of trypsin. Trypsin digests proteins into smaller peptide and amino acid molecules. However, since trypsin itself is a protein, it is capable of digesting itself, which is known as autolysis. If we assume that the amount of trypsin present in a dusted fresh print is many times more than the amount of protein present in the original fingerprint, and if we further assume that trypsin digests its own proteins at approximately the same rate as it does the fingerprint protein, we must conclude that probably most of the observed increase in ninhydrin color for fresh prints is due to amino acids generated from the trypsin itself rather than from the fingerprint protein.

For the enhancement of old fingerprints, however, we rule out autolysis as the dominant mechanism over enzymatic activity on the fingerprint residue for several reasons. For fresh prints, zero incubation time samples yield enhancement (as described earlier) because the trypsin (which reacts with ninhydrin) preferentially sticks to the fingerprint residue. For old prints, there is no preferential sticking, as shown in Fig. 2. The fact that old prints are generally not amenable to dusting is, incidentally, well known. Enhancement for old prints should thus be accompanied by strong background if enzyme autolysis is the dominant enhancement mechanism. Indeed, in our earlier study [*2*], strong background was observed with no preferential enzyme adherence to the fingerprint, the background arising from impurities (or autolysis products) reacting with ninhydrin. On the other hand, the Sigma Type III lyophylized trypsin produced enhancement without such background. Further, the reused trypsin in which enzymatic activity was lost (presumably because of autolysis) produced negligible enhancement of detail of old prints. The observed temperature and moisture effects are consistent with either autolysis or enzymatic activity. Our results indicate that the latter is dominant in producing enhancement of old prints. However, our experiments cannot quantitatively assess the relative contributions of the two mechanisms. From the pragmatic standpoint, this distinction is of no importance.

The fact that trypsin can be used to achieve enhancement of already developed ninhydrin prints provides the opportunity to bring out detail in weak prints. Even though the enhancement is not as great after ninhydrin development as it is before the application of ninhydrin, it may be practical to use trypsin after ninhydrin development only, except in special circumstances. Trypsin is rather specific in its hydrolysis of proteins, and thus probably not the best enzyme for fingerprint development. We are currently in the process of evaluating a range of enzymes that are potentially better than trypsin.

### Zinc Chloride or Cadmium Nitrate for Enhancement of Fingerprint Development

The purple product of the reaction of ninhydrin with amino acids of fingerprint residue can be converted to an orange or red form by subsequent treatment with zinc chloride or cadmium nitrate, respectively. The orange or red products do not lead to improved visualization in room light. Indeed, developed prints appear fainter, and, if excessively treated with the metal salts, may show loss of detail. However, under examination by blue-green laser, the orange or red

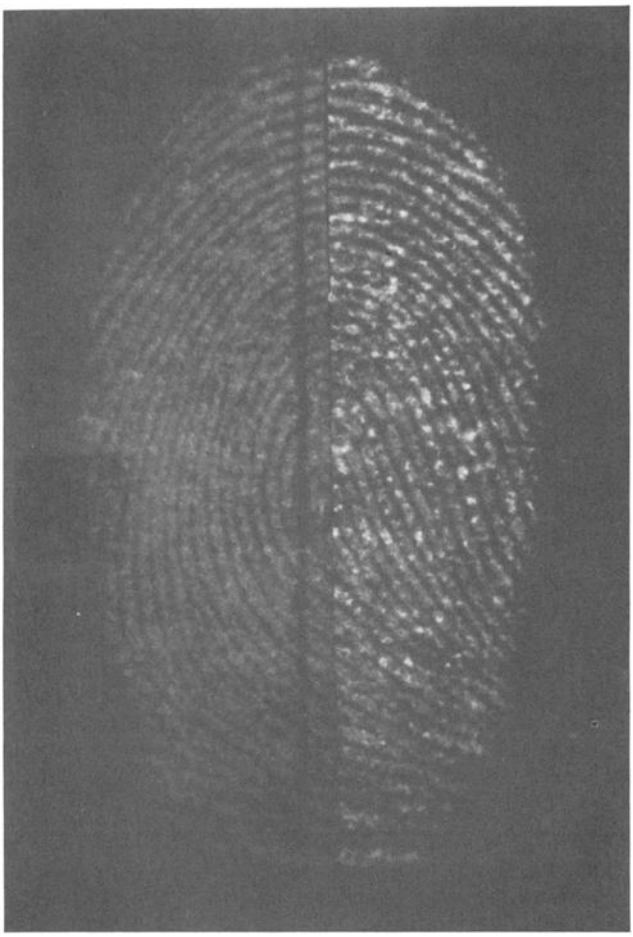

FIG. 3—*Latent print under all lines blue-green Ar-laser excitation. Left half treated with ninhydrin/* $Cd(NO_3)_2$, *right half with ninhydrin/* $ZnCl_2$.

products show strong fluorescence, thus strongly enhancing fingerprint detectability. Under all lines of blue-green or 514.5-nm Ar-laser excitation, the fluorescence obtained with $ZnCl_2$ is generally stronger than that obtained with $Cd(NO_3)_2$ (see in Fig. 3). The $ZnCl_2$ treatment after ninhydrin is best matched to the 488-nm line of the Ar-laser and produces a yellow fluorescence (orange when high concentrations are present), whereas $Cd(NO_3)_2$ after ninhydrin shows an absorption maximum at about 510 nm, which makes the 510-nm Cu-vapor laser line or the 514.5-nm Ar-laser line very suitable for excitation. $Cd(NO_3)_2$ treatment produces an orange fluorescence. Even though $ZnCl_2$ generally yields the stronger fluorescence, $Cd(NO_3)_2$ may at times be advantageous if contrast with background fluorescence is a problem. $Cd(NO_3)_2$ may be more suitable to use with frequency-doubled Nd:YAG lasers than $ZnCl_2$. Further spectroscopic optimization can be achieved if ninhydrin analogues are used instead of ninhydrin [5].

### *Ninhydrin and Metal Salt Solutions*

The ninhydrin solution with which latent prints are to be developed must satisfy conflicting requirements. On one hand, it is desirable to deliver as much ninhydrin as possible to the fingerprint to react with amino acids. On the other hand, the polar solvents for ninhydrin dissolve amino acids and thus tend to smudge out detail. Moreover, they cause ink running on paper. One thus wants a fast-drying solvent. Of the polar solvents that are candidates from a practical standpoint, methanol is one of the most volatile ones. By itself, however, it is not sufficiently volatile. Thus, ninhydrin is first dissolved in the methanol to near saturation, about 2.7*M*. The solution is then added to the solvent freon, which functions as a volatile carrier. Although we have not optimized the methanol:freon ratio, we find a 1:5 ratio quite suitable. Fingerprint smudging and ink running do not constitute a problem, and latent prints are found to develop fully in ambient conditions (no heating with moisture) in about 4 to 5 h.

To optimize the ratio of $ZnCl_2$ (or $Cd(NO_3)_2$) to the ninhydrin/amino acid reaction product, a solution of this product was titrated with $ZnCl_2$ in amounts increasing in steps of 10% while monitoring the absorbance at 490 nm. The highest optical density was obtained with about 80% of the ninhydrin molarity. We therefore use 2.7*M* $ZnCl_2$ or $Cd(NO_3)_2$ solutions in methanol and dilute with freon in 1:5 ratio, as with ninhydrin.

### *Optimization of Reaction Conditions*

We have assessed the effect of atmospheric humidity on the rate of formation of the $Zn^{++}$ or $Cd^{++}$/ninhydrin/amino acid product. Table 1 shows the effects of temperature and relative humidity on formation of the fluorescent Zn or Cd coordination compound with the purple ninhydrin/amino acid reaction product [5]. Clearly, high relative humidity and somewhat elevated temperatures (about 40°C) expedite the reaction. A sample incubated at 0% relative humidity and 45°C showed no reaction. This shows that ambient humidity is essential. Addition of water directly to the metal salt/methanol/freon solution is deleterious to fingerprint development. Also, since methanol and freon are not readily miscible, the solutions need to be shaken vigorously just before spraying to maintain an adequate emulsion. Latent prints developed by $ZnCl_2$ or $Cd(NO_3)_2$ treatment and laser examination need to be photographed fairly promptly because the metal salts absorb moisture from the air, which ultimately causes smudging of detail. Since a number of chemicals used in fingerprint work are potential carcinogens or are toxic, the use of gloves of good quality should be routine, and treatments should generally be done in a fume hood.

### *Acknowledgment*

This material is based upon work supported by the National Science Foundation under Grant CHE 83 13527.

TABLE 1—*Effects of temperature and humidity on reaction of $ZnCl_2$ and $Cd(NO_3)_2$ with ninhydrin/amino acid reaction product.*

| Incubation Conditions | | Development | |
|---|---|---|---|
| Temperature, °C | RH, % | Time | % of Full Dev.[a] |
| | | $ZnCl_2$ | |
| 23 | 8 | 3 h | 0 |
| 23 | 30 | 1 h | 60 |
| 23 | 80 | 8 min | 90 |
| 37 | 85 | 3.5 min | 100 |
| 60 | 65 | 1.5 min | 100 |
| | | $Cd(NO_3)_2$ | |
| 23 | 8 | 2 h | 0 |
| 23 | 35 | 2 h | 20 |
| 23 | 75 | 10 min | 90 |
| 37 | 90 | 1.5 min | 100 |
| 60 | 57 | 1 min | 100 |

[a]Visual estimate.

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